Proceedings of the 29th Annual Aquatic Toxicity Workshop: October 21-23, 2002, Whistler, British Columbia

Comptes rendus du 29e atelier annuel sur la toxicité aquatique du 21-23 octobre 2002, Whistler, Colombie Britannique

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PREFACE / PREFACE

The 29th Annual Aquatic Toxicity Workshop was held at the Fairmount Chateau Whistler in Whistler, British Columbia, October 21-23, 2002. The Workshop included 2 plenary presentations, 136 platform and 86 poster papers. Total attendance was 401.

This Workshop was one of a continuing series of annual Workshops in Canada on aquatic and environmental toxicology, covering topics from basic aquatic toxicology to applications in environmental monitoring, setting of regulations and guidelines, and the development of sediment and water quality criteria. These Workshops emphasize an informal exchange of ideas and knowledge on the topics among interested persons from industry, governments and universities. They provide an annual focus on the principles, current problems and approaches in aquatic toxicology. These Workshops are run by an incorporated National Steering Committee, and the Proceedings are published with the support of the Department of Fisheries and Oceans.

Le 29e atelier annuel sur la toxicité a eu lieu Fairmount Chateau Whistler, Whistler, British Columbia, au 21-23 octobre 2002. Le atelier a donné lieu a 2 communication lors de séances plénières, 136 exposés d'invités d'honneur 86 communications par affichage. 400 personnes ont assisté au atelier.

Le atelier a premis de poursuivre les discussions tenues annuellement au Canada sur la toxicologie aquatique et l'écotoxicologie. Ces atelier annuels organisés par un comité national constitué légalement réunissent des représentants des secteurs industriels, des administrations et des universités que le domaine intéresse. Ces derniers y échangent des idées et des connaissances sur les notions fondamentales de la toxicologie aquatique, mais aussi sur son application pour la surveillance de l'environnement, l'élaboration de lignes directrices et de règlements, et la définition de critère pour les sédiments et pour la qualité de l'eau. Ils passent également en revue les principes de la spécialité, de même que les questions d'actualité et les méthodes adoptées dans le domaine. Les comptes rendus sont publiés l'aide du ministère des Pêches et Océans.

EDITORS COMMENTS / REMARQUES DES EDITEURS

This volume contains papers, abstracts or extended abstracts of all presentations at the Workshop. An author index and list of participants are also included. The papers and abstract were subject to limited review by the editors but were not subjected to full formal or external review. In most cases the papers are published as presented and therefore are of various lengths and formats. Comments on any aspects of individual contributions should be directed to the authors. Any statements or views presented here are totally those of the speakers and are neither condoned or rejected by the editors. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Ces comptes rendus sont publiés en deux volumes, en raison de leur longeur, ils renferment le texte intégral ou le résumé de toutes les communications présentées aux ateliers. Un index des auteurs et une liste des participants sont aussi inclus. Les communications et les résumés ont été revus sommairement par les éditeurs, mais ils n'ont pas fait l'objet d'une revue exhaustive en bonne et due forme ou d'une revue indépendante. La lonueur et la forme des communications varient parce que ces derniéres sont pour la plupart publiées intégralement. On est prié de communiquer directement avec les auteurs pour faire des remarques sur le travaux. Toutes les déclarations et opinions paraissant dans le présent rapport sont celles des conférenciers; elle ne sont ni approuvées, ni rejetées par les éditeurs. La mention de marques de commerce ou de produits commercialisés ne constitue ni une approbation, ni une recommandation d'emploi.

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PLENARY SESSION / SÉANCE PLÉNIERE

Emerging Toxicological Issues in the Pacific Northwest

Moderator: G.C. van Aggelen

Speakers

David Marshall, Fraser Basin Council
Honourable Michel Bourassa, Court of the Northwest Territories
Chief Bill Williams, Telasemkin First Nations
Bob Baker, Telasemkin First Nations

TECHNICAL SESSIONS / SÉANCES TECHNIQUE

Pulp and Paper Environmental Effects Monitoring

Session Co-chairs: K. Hedley and B.K. Firth

National Assessment of Pulp and Paper Mill Environmental Effects Monitoring: Overall Patterns of Effects. R.B. Lowell[1,2], J.C. Culp[2], L.C. Grapentine[3], M.E. McMaster[3], K.R. Munkittrick[4], T.B. Reynoldson[5] and S.C. Ribey[1]. [1]Environment Canada, National EEM Office, Ottawa, ON; [2]Environment Canada, National Water Research Institute, Saskatoon, SK; [3]Environment Canada, National Water Research Institute, Burlington, ON; [4]Department of Biology, University of New Brunswick, St. John, NB; and [5]Acadia Centre for Estuarine Research, Acadia University, Wolfville, NS.

The Environmental Effects Monitoring Program tracks the field effects of pulp and paper mill effluents across the country. We have analyzed the results to date to help interpret the type and magnitude of effects on benthic invertebrates and fish in freshwater and marine receiving waters. Using a newly developed combination of meta-analytical and multivariate techniques, it was possible to quantitatively integrate the invertebrate and fish data and to identify a number of national response patterns in areas exposed to pulp mill effluent. These national patterns were fully consistent with smaller scale responses described in the literature, allowing us to interpret the results within the context of known mechanisms of effects.

The most common effluent-associated effect on benthic invertebrates was one of mild to moderate eutrophication, although there was evidence of more pronounced eutrophication, toxicity, or smothering effects at some mills. The most common effluent-associated effect on fish was a combination of nutrient enrichment and metabolic disruption, although other response patterns were also observed, including differing degrees of nutrient enrichment versus limitation. Multivariate analyses of the meta-analytical output revealed good agreement between the benthic invertebrate and fish results and, at the same time, illustrated how each core endpoint tracked a different aspect of the overall response to effluent exposure.

The Environmental Effects Monitoring TRIAD Index or How to Integrate the Results from the Fish Survey, the Benthic Invertebrate Survey and the Sublethal Toxicity Tests for the Québec Pulp and Paper Mills Cycle 2 EEM Studies. R. Chabot and A. Willsie. Environnement Canada, Direction de la protection de l'environnement, Montréal, Qc.

As part of the second cycle of the Environmental Effects Monitoring (EEM) Program conducted under the Canadian Pulp and Paper Effluent Regulation, 46 EEM studies were conducted in 1998 to 1999 by Québec pulp and paper mills. The objective of this communication is to show the usefulness of an approach based on the weight of evidence concept that integrates the results from three main components in EEM namely: fish survey, invertebrate community survey and sublethal toxicity tests. The EEM triad index is a tool used to assess the effects of mill effluents on the receiving environment. It combines the information from each mill thus enabling easier communication of results; it also helps to compare mills among themselves and to evaluate temporal changes. The EEM triad index is a composition of the transformed results of all the endpoints in each component of the EEM program.

A score is attributed according to the amplitude of the effect for each component. A score of 5 represents a very important impact on the receiving environment while a score of 1 means no effects were observed for that component. A specific EEM triad index value (between 1 and 35) is then attributed to a specific combination of the 3 components' scores. Although the implementation of secondary treatment in 1995 contributed to major improvement of the habitat quality of many receiving waters, the Cycle 2 results show that a majority of mills still have an impact on fish or their habitat. The integration approach proposed in this presentation looks very promising.

Assessing Effects of Pulp Mill Effluent on Benthic Invertebrates with Stream Mesocosms on the Saint John River, Edmundston, NB. N.E. Glozier[1.] J.M. Culp[2,3], K.J. Cash[3], R.B. Brua[3] and C.S. Wood[4]. [1]PNR-Environment Canada, Ecological Research Division, Saskatoon, SK; [2]Department of Biology, University of New Brunswick, Fredericton, NB; [3]Environment Canada, National Water Research Institute, Saskatoon, SK; and [4]Noranda Inc., Centre de Technologie, Pointe-Claire, QC.

On-site stream mesocosms can be used to integrate several tiers of monitoring for riverine food webs, especially when complex exposure dynamics or investigation of cause requires evaluation. To determine the effects of pulp mill effluent (0, 5, 10% v/v PME) on benthic invertebrate communities of the Saint John River, two experiments using mesocosms were conducted (fall 1999 and summer 2000). The NWRI benthic invertebrate mesocosm facility consists of a series of partial flow-through, modular wet tables with 5 to 8 replicate streams. Enrichment effects of nutrient additions from low PME concentrations often override the effects of contaminants within the effluent. This result was observed in both field experiments with increased accrual of periphyton biomass along with shifts in diatom community structure. Benthic invertebrate community structure also showed significantly shifts (Bray-Curtis Similarity Index and multivariate analyses) even though PME addition did not appear to impact other benthic invertebrate endpoints (i.e., density, richness, or diversity). By accounting for emerged insects in the mesocosms in 2000, we found higher densities of Diptera in 5% PME relative to 0% PME and lower Ephemeroptera densities. These results reinforce the need to examine both compositional and functional endpoints in the assessment of environmental impacts and are consistent with national trends observed in Environment Canada's EEM Cycle 2 assessment.

Getting Outside the Box: To Go Where No EEM Has Gone Before. N. Munteanu and G.P. Thomas. G3 Consulting Ltd., Burnaby, BC.

Preliminary results of meta-analyses by Environment Canada on Cycles 1 and 2 of Pulp and Paper EEM have been released and Cycle 3 is currently underway. National trends regarding EEM findings point to several challenges and the need for innovation. Investigations in any subsequent cycle will ultimately require additional tools and methods to further discern magnitude and effect as well as accurately assess cause and effect (and possibly significance). Challenges faced by current and subsequent EEM cycles across Canada include: [1] discerning current from historical effects, [2] differentiating mill effluent-effect from other mill-related effects (e.g., log-handling, others), [3] accurately defining effect and significance (as opposed to "difference") and assigning cause, and [4] better defining enrichment-effects, found nationally. This paper discusses several tools not typically applied under EEM, but which have proven extremely useful in environmental assessment research for decades. These include: periphyton (component); fitness parameters and biotic indices (endpoints); revised Sediment Quality Triad (methodology). Case studies (i.e., EEM Cycle 3 investigations) and examples are also discussed to explore the advantages and limitations concerning these potentially useful tools in EEM.

Evenness and Diversity Indices in Environmental Effects Monitoring Invertebrate Community Surveys. M.D. Paine. Paine, Ledge and Associates (PLA), North Vancouver, BC.

Evenness and diversity are measures of richness (number of taxa; S) relative to total abundance (N). S and N will usually be positively correlated unless pollution and effects are severe. Most evenness and diversity indices are not recommended, because unwarranted assumptions are made about the S-N relationship and values are dependent on N and rarely comprehensible or meaningful. Simpson's index λ is a simple and comprehensible univariate measure of S relative to N. λ provides the probability that two organisms drawn randomly from a population will belong to the same taxon. The metal mine EEM guidance document recommends a version of λ which adjusts for the maximum possible value for any given S. Consultants conducting EEM programs should be aware that their favourite software may provide a different version of λ. Never report and analyze an index value unless you know how it is calculated and can explain what it means. I use residuals from log-log regressions of S versus N for the actual data as measures of evenness or diversity. The regression slope will usually be ≈ 0.3, and S may be nothing other than a cube root transformation of N. The spatial scale at which S is measured (single samples? pooled samples from a station or area?) is also important for analyses and interpretation.

A Tiered Framework for Investigation of Cause in Pulp and Paper Environmental Effects Monitoring. L.M. Hewitt[1], M.G. Dubé[2], J.M. Culp[2,3], D.L. MacLatchy[2] and K.R. Munkittrick[2]. [1]Environment Canada, National Water Research Institute, Burlington, ON; [2]Environment Canada, National Water Research Institute, Saskatoon, SK; and [3]Department of Biology, University of New Brunswick, Fredricton, NB.

Environmental Effects Monitoring (EEM) has completed two cycles in the pulp and paper sector and is initiating its first cycle for metal mining. We propose a framework here that utilizes a tiered approach to investigating cause using pulp and paper as a model. Within the pulp and paper model we have incorporated several ongoing research projects that are currently characterizing sources and identities of bioactive chemicals that originate from papermaking. A determination of cause ultimately ends with the confirmation of individual chemicals present in a effluent that elicit the responses observed in conducting EEM studies. It is our contention that this level of detail is not required at all sites where differences have been noted, nor is it economically feasible. We propose that the depth to which an investigation of cause is conducted be determined on a site-specific basis which is decided upon by stakeholders. Our framework consists of 3 tiers, each of which provides more information on the sources and identities of the responsible compounds. The first tier of our approach consists of confirmation that the source of the effect is indeed the discharger. The second tier involves investigating individual process effluents within the industrial facility to determine their relative contributions to the effects that have been observed. The final tier involves isolating and characterizing the individual chemicals associated with the responses using techniques such as bioassay-directed fractionation. This framework can be used in a stakeholder decision-making process to determine the extent of the investigation. Intensive research projects utilizing the approaches within this framework at selected sites will ultimately provide a database of causative processes and/or substances that will be useful in conducting investigations at other sites.

Immunomodulation in Blue Mussels (*Mytilus edulis*) Exposed to a Pulp and Paper Mill Effluent in Eastern Canada. S.D. St-Jean[1], S.C. Courtenay[1] and W.R. Parker[2]. [1]Department of Fisheries and Oceans, Gulf Fisheries Centre, Moncton, NB; and [2]Environment Canada, Environmental Protection Branch, Fredericton, NB.

Blue mussels (*Mytilus edulis*) were caged at three sites situated at increasing distance from the point of discharge of a pulp and paper mill effluent from July to October 1998. Two additional cages were deployed: one inside and one at the mouth of the adjacent industrialized Pictou Harbour. After 90 days exposure, we measured growth and survival, haemocyte counts (HC), phagocytic activity (PA), lysosome retention (LR) and bacterial clearance (BC). No growth and survival differences were noted for mussels between the five cages. Mussels from three cages showed similar difficulty in clearing bacteria; the cage inside the harbour, the cage nearest to the pulp mill effluent and the cage receiving a mixture of both effluents. These mussels also showed the highest heavy metal burdens and conversely, the cage showing the most rapid clearance, outside both effluents, also showed the lowest heavy metal burden. Mussels caged in the pulp mill effluent showed lower PA and LR and higher mortality during the immune challenge than other mussels. These results suggest that immunological biomarkers may be useful, and more sensitive, in short-term monitoring studies than growth and survival. This could be of importance for future programs such as environmental effects monitoring or environmental assessment of locations potentially exposed to anthropogenic inputs.

Effects of Kraft Pulp Mill Effluent Contamination on the Age, Growth Rate and Elemental Composition of Mussels (*Mytilus trossulus* and *Mytilus edulis*). R.C.H. Wilson [1], J.A.J. Thompson[1] and C.A. Richardson[2]. [1]2WE Associates Consulting Ltd., Victoria, BC; and [2]School of Ocean Sciences, University of Wales - Bangor, Menai Bridge, Anglesey, UK.

The age and growth rate of the blue mussel *Mytilus trossulus* living close to the Crofton, Vancouver Island, pulp mill were determined from the patterns of wide and narrow microgrowth increments in acetate peels of shell sections. The age (<6 years) and growth of mussels close to the Crofton outfall were similar to those from a reference site on Salt Spring Island. An extended mill shutdown period could not be distinguished in the growth record. ICP-MS analysis of the shells indicated Pb, Cu, Zn and Mn were significantly lower in shells close to the discharge.

Shells of *Mytilus edulis* transplanted into cages along a discharge gradient from the pulp mill in Pictou harbour (Nova Scotia), showed significantly lower levels of Pb, Zn and Mo compared with pre-exposure shells. Ni and Mn were significantly elevated in the caged mussels, while levels of Cu were generally indistinguishable from the pre-exposure group. The potential use of growth bands, the significance of elemental differences in the shells, and possible causes of the observed elemental changes will be discussed in relation to monitoring of wood pulp mill waste in Canadian coastal waters. We think that depletion of certain metals in the shell provides a marker of exposure to pulp and paper mill effluents.

Using Caged Mussels to Monitor Dioxins and Furans in the Kennebec River, Maine.

M.H. Salazar and S.M. Salazar. Applied Biomonitoring, Kirkland, WA.

Abstract

During the summer of 2000, a 53 day pilot study was conducted in the Kennebec River, Maine to determine whether caged freshwater mussels (*Elliptio complanata*) would be a reasonable surrogate for resident fish to assess upstream and downstream exposures of dioxins and furans associated with pulp and paper mill effluents. Caged mussels were deployed 13 miles upstream and 11 miles downstream from a pulp and paper mill. Mussels were deployed at these locations because they were the closest areas where fish could be collected due to the limitations of fish sampling and dams on the river. Mean total dioxin/furan concentrations in mussel tissues increased from below detection before deployment to 4.33 and 4.67 ng/kg-ww (parts-per-trillion) at the upstream and downstream stations after deployment. There was no statistically significant difference between upstream and downstream total dioxin/furan concentrations. More individual dioxin/furan congeners were measured in mussel tissues from both upstream and downstream locations than in either semi-permeable membrane devices (SPMDs) or fish tissues collected during the same time period. Advantages and disadvantages of caged mussels, natural fish populations, and SPMDs will be discussed along with the benefits of a gradient sampling design relative to using only upstream and downstream comparisons where the fish could be caught by angling.

Background

The State of Maine Department of Environmental Protection (DEP) has expressed concern regarding the ability to detect statistically significant differences in chemical exposure when comparing upstream and locations from pulp and paper mills due to declining tissue concentrations of dioxins and furans in fish. These comparisons are important because environmental regulations do not allow significant differences in upstream versus downstream exposures associated with those effluents. Academic and public environmental groups and mill representatives have all expressed concerns about using the fish test for this purpose and the fish test has limited support outside DEP. Many have identified problems with monitoring indigenous fish populations for upstream/downstream comparisons at mill sites, including uncertainty associated with mobility, accumulation from other sources, accumulation from previous mill discharges sequestered in sediments, and the inability to collect fish near the mill discharge. One environmental group supported and advocated the caged mussel pilot study anticipating that concerns regarding fish monitoring could be eliminated by using a surrogate, such as caged mussels, that could be deployed closer to the mill discharge where fish could not be collected.

DEP is responsible for developing a monitoring program to assess the nature and extent of dioxin and furan contamination in the waters and fisheries of the state but many have suggested that they have yet to develop an appropriate test. Maine has adopted the most stringent environmental regulations for dioxins in the US, and the primary objective of the dioxin/furan monitoring program is to assess potential ecological and human health effects by measuring chemical exposure in fish tissues. Interestingly, Environment Canada has adopted the opposite approach and focused on measuring effects in fish or suitable surrogates. Caged mussels and mesocosms have been accepted as alternatives the adult fish survey in required Environmental Effects Monitoring (EEM) at pulp and paper mills in Canada. A secondary objective of dioxin monitoring in Maine is to document the status and trends in of dioxin/furan exposures, evaluate progress in reducing environmental concentrations by compliance with existing regulations, and the need for even more stringent regulations. The third, and most specific objective is to determine if kraft pulp mills are discharging dioxins or furans into the rivers of Maine. A state law enacted in 1997 prohibits such discharges and requires compliance by December 31, 2002. In practice, environmental exposures of dioxins and furans estimated by measuring concentrations in fish tissues or some surrogate, cannot be higher downstream of a pulp mill discharge than upstream. This is commonly referred to as the "above/below" test.

In 2000, DEP continued development of an appropriate "above/below" fish test, but as dioxin and furan concentrations decline, there were concerns that the existing monitoring approach may not be sufficiently sensitive to detect statistically or environmentally significant differences in exposure to properly evaluate compliance with the 1997 state law. Many believe that limitations of the fish test may preclude a scientifically or legally defensible use of the fish test in its current form. Instead of considering methods such as the caged mussels or SPMDs as surrogates for fish, it might be more appropriate to consider the use of fish in addition to surrogate tests in a weight-of-evidence approach. Although concentrations of dioxins and furans measured in fish tissues were higher below than above pulp mill discharges in 1999, questions remain about the suitability of fish as effective monitors. These questions are related to: [1] The mobility of fish and where exposure to dioxins and furans actually occurred, [2] Whether fish accumulated dioxins and furans from sediment or food that was contaminated from previous, rather than recent mill discharges, and [3] When exposure and accumulation in collected fish occurred. In response to some of these questions, DEP modified the 2000 fish monitoring program to include measuring dioxins and furans in tissues of caged mussels and in lipids of SPMDs as potential surrogates for monitoring dioxins and furans in fish tissues.

Caged freshwater bivalves have been used to monitor dioxins and furans associated with pulp and paper mill effluents in Finland and for similar chemicals such as PCBs in Canada for approximately 20 years. Environment Canada has recently adopted caged bivalve monitoring as an alternative to the required adult fish survey in their EEM program for pulp and paper mills in Canada. Standardized protocols have been adopted by the American Society for Testing and Materials (ASTM) for conducting caged bivalve studies, and a standard guide appeared for the first time in the 2001 ASTM Annual Book of Standards. A revised version will also appear in the upcoming Standard Methods for the Examination of Water and Wastewater. Caged bivalves are a potentially powerful tool because of their ability to quantify exposure and effects over space and time. In situ studies with caged bivalves could complement and help establish links between various elements of the existing DEP monitoring program through the use of tissue chemistry and mussel growth measurements. This approach could also help reduce uncertainty in the current approach and answer questions within government, industry, and the public regarding chemical exposure and biological effects associated with pulp mill effluents. It is also consistent with the ecological risk assessment process of characterizing exposure through bioaccumulation and characterizing effects through mussel growth rates. As mentioned previously, Environment Canada has focused on characterizing effects in its EEM program while the State of Maine has focused on characterizing exposure. The ecological risk assessment paradigm suggests equal emphasis on exposure and effects in a more balanced approach.

Methods

Freshwater mussels (*Elliptio complanata*) were collected from Nequasset Lake, a relatively clean lake within the Kennebec watershed in Woolwich, Maine, caging individuals of a minimum size range, and transplanting them 13 miles upstream and 11 miles downstream from a pulp an paper mill in the Kennebec River (Fig. 1). The mill is located in Hinckley, approximately 30 miles north of Augusta, Maine. DEP insisted on using only one upstream and one downstream station, i.e., locations closest to the mill where fish could be collected, so that mussel data could be directly compared with fish data. They did not allow us to place caged mussels any closer to the mill than 13 miles upstream and 11 miles downstream, even though there were extra cages that could have been used. This precluded a thorough evaluation of the caged mussel methodology.

Ten cages with 36 mussels each were deployed for 53 days at these two locations in accordance with the upstream/downstream test paradigm. Figure 1 also shows our proposed experimental design with only three cages upstream and three cages placed at each of five downstream stations in a gradient design. This was our recommended approach and the one advocated in the ASTM Standard Guide for conducting *in-situ* bioassays with caged marine, estuarine, and freshwater mussels. After retrieval, the whole soft tissues of mussels were analyzed for dioxins and furans, percent lipids, and percent moisture. Percent lipids were measured as another indicator of animal health and to normalize the measurements on a lipid basis. Percent moisture was another indicator of animal health and used to normalize the data on a dry weight basis. Percent survival and multiple growth metrics were used as the primary indicators of animal health.

Results

Survival and growth of caged mussels indicated they were all in adequate health to accumulate dioxins and furans if present. Mean concentrations of total dioxins/furans in mussels increased from below detection at the beginning of the test to 4.33 and 4.67 ng/kg-ww at the upstream and downstream stations, respectively, at the end of the test (Fig. 2). Concentrations were higher downstream than upstream, but the difference was not statistically significant difference between upstream and downstream total PCDD/PCDF concentrations at the end of the test. More individual dioxin/furan congeners were measured in mussel tissues from both upstream (15 congeners) and downstream (13 congeners) locations than in SPMDs (11 and 12 congeners) or fish tissues (4 and 5 congeners) (Figs. 3A,B). We believe these results are encouraging with respect to using caged mussels as a surrogate for fish, particularly since the downstream station was located 11 miles from the mill and mussels still accumulated both dioxins/furans. The gradient design could have proven the existence of dioxins and furans closer to the mill if they were really being discharged by the mill.

The concentration of total dioxins/furans in fish tissues was significantly higher 11 miles downstream (4.19 ng/kg-ww) than 13 miles upstream (2.76 ng/kg-ww) of the mill (Fig. 4). These data suggest that fish are better able to detect differences in dioxin and furan exposure than mussels or SPMDs, and the existing fish monitoring approach is appropriate. However, on a lipid-normalized basis, concentrations of total dioxins/furans in fish collected at upstream and downstream stations are not significantly different. As with the data for SPMDs, the lipid-normalized concentrations for fish are higher upstream than downstream, but not significantly different. These data reinforce the significance of the important questions mentioned earlier regarding where the fish were exposed to

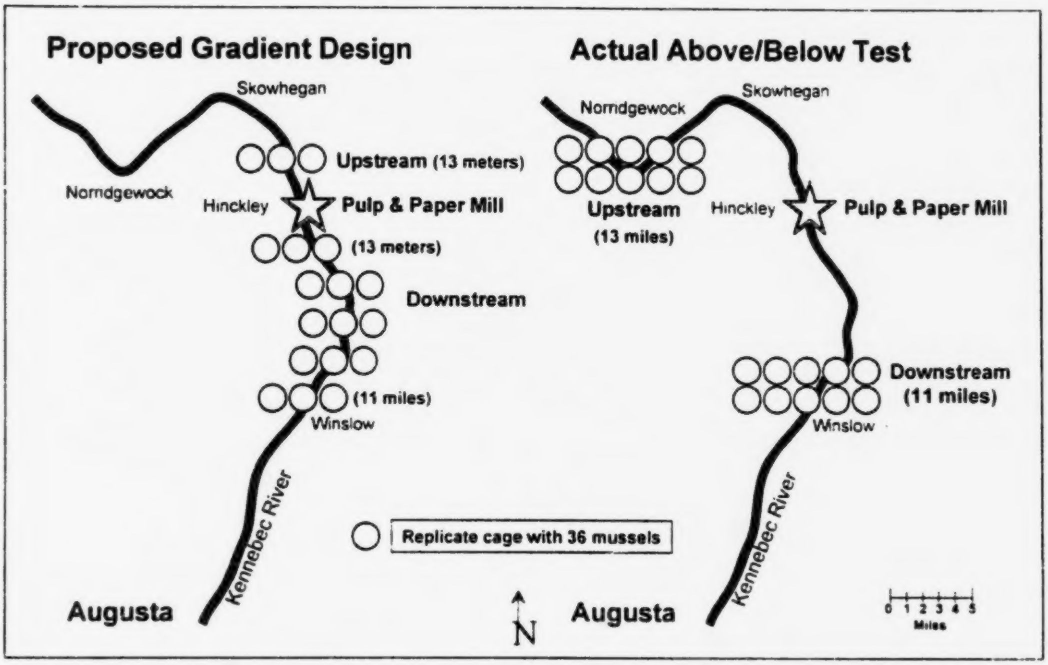

Proposed Gradient Design
Actual Above/Below Test
Skowhegan
Norridgewock
Upstream (13 meters)
Hinckley
Pulp & Paper Mill
(13 meters)
Downstream
(11 miles)
Winslow
Kennebec River
Replicate cage with 36 mussels
Augusta
N
Norridgewock
Skowhegan
Upstream
(13 miles)
Hinckley
Pulp & Paper Mill
Downstream
(11 miles)
Winslow
Kennebec River
Augusta
0 1 2 3 4 5
Miles

Figure 1. Site map showing proposed experimental design versus stations where caged mussels were deployed.

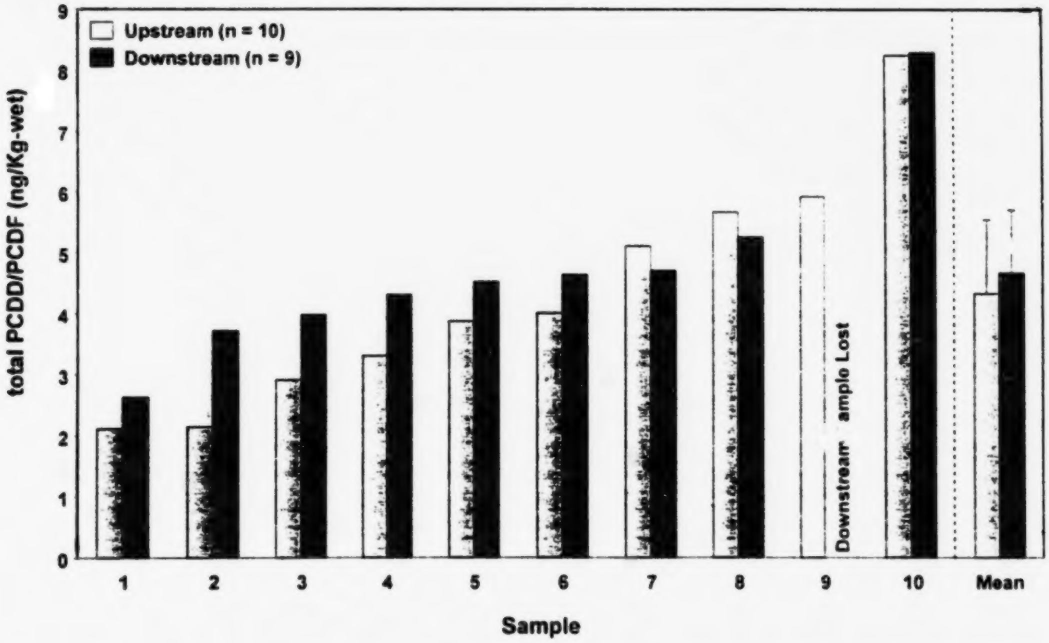


Figure 2. Total dioxins and furans in caged mussels from upstream and downstream stations.

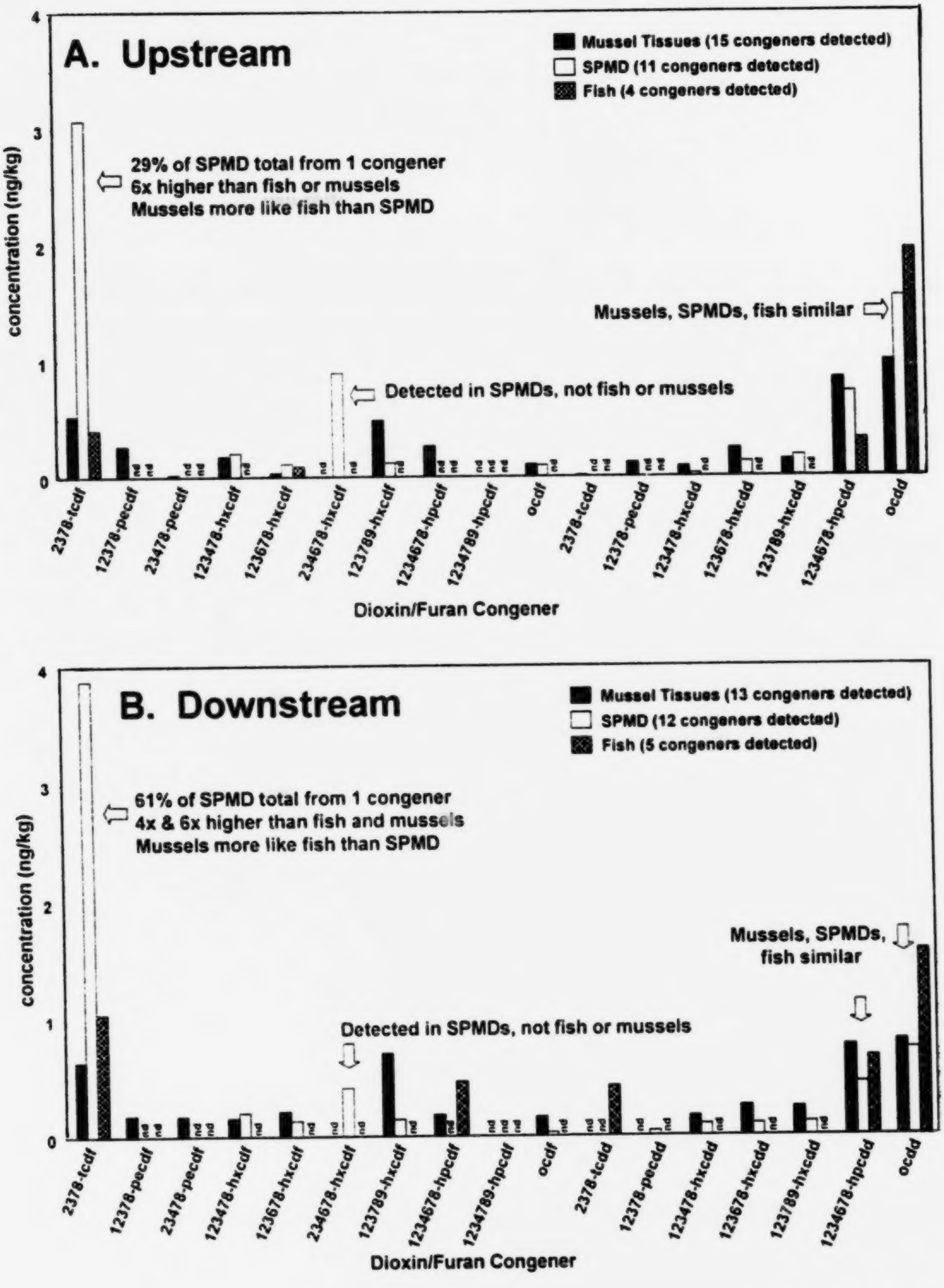


Figure 3. Mean concentration (ng/kg) of individual congeners measured in mussel tissues, SPMDs, and fish tissues. A = Upstream dioxin station; B = Downstream dioxin station. ND = not detected.

dioxins and furans, whether they accumulated dioxins and furans from sediment or food that was contaminated from previous, rather than recent mill discharges, or how long ago exposure and accumulation occurred.

Figure 4 also shows that total dioxin and furan concentrations in caged mussel tissues were higher downstream than upstream on both a lipid-normalized and a non-lipid normalized basis, although the differences were not statistically significant. Total dioxins and furans in SPMDs were higher upstream and downstream on both a lipid-normalized and a non-lipid-normalized basis although these differences were not statistically significant either. However, the SPMDs consistently demonstrated higher concentrations of dioxins/furans upstream than downstream. The fish demonstrated higher concentrations downstream when the data were not lipid normalized and higher concentrations upstream when the data were lipid normalized.

There was also much greater uncertainty in the SPMD data when compared to the mussel and fish tissue chemistry data. Nearly 40% of the congeners in mussel tissues were present at concentrations exceeding the detection limit, compared to approximately 20% for fish, and less than 10% for the SPMDs (Fig. 5). This is based on results of congener-specific analyses that yielded 153 values for mussel tissues, 81 values for fish tissues, and 77 values for SPMDs. Some results for both the mussel tissues (<10%) and SPMDs (<40%) were reported at concentrations greater than zero, but less than the detection limit. For the SPMDs, these concentrations were generally at least one order of magnitude lower than the detection limit. Plots of the ratio of measured concentrations of the individual congeners divided by the method detection limit for each congener for mussels, SPMDs, and fish show the greater uncertainty in the SPMD data (Fig. 5). Only 10 of the measured values (12%) for SPMDs are above the detection limit, only one value within 50% of the detection limit, and the rest of the values were between 0.4% and 29% of the detection limit. These reported concentrations were estimated from the calibration curve of the analytical instrument, but have the greatest uncertainty because they are so far away from the instrument detection limit. These data suggest that the extremely low measured concentrations and the large number of non-detects from samples collected 13 miles upstream and 11 miles downstream are not reliable indicators of dioxin/furan exposure, and that there may have been an analytical problems associated with these data. A recent quality assurance/quality control (QA/QC) review has suggested a methodological problem at the laboratory conducting the analyses, and when extra fish samples were analyzed, the concentrations were significantly higher. The original data were questioned when the reported concentrations were significantly lower than the previous year and there were no process changes at many of the mills that were being monitored.

Discussion

Collectively, the congener-specific data which showed detection of more congeners in mussels than SPMDs and fish, results that showed higher concentrations in mussels downstream than upstream (on both a lipid-normalized and non-lipid-normalized basis), and the larger number of samples above the detection limit suggests that mussels were better dioxin/furan indicators than SPMDs or fish. The most important question to be asked may be whether or not the fish data are believable, particularly given their ability to move and accumulate dioxins and furans through other exposure pathways. Just because the fish test satisfied the requirements of the above/below test and implicated the mill does not mean that these data represent "real-world" conditions at the sampling locations located 13 miles upstream and 11 miles downstream. This appeared to be one of the most important considerations for DEP in evaluating the suitability of caged mussels as a surrogate test. These questions, as well as concerns regarding upstream and downstream comparisons, can be addressed, at least in part, by using a weight of evidence approach. We carefully scrutinized the total concentrations of dioxins and furans measured in each test matrix (mussels, SPMDs, fish), the lipid normalized concentrations, and the concentrations of individual congeners.

Although the fish appeared to be the most suitable monitoring tool based on the ability to detect statistically significant differences between upstream and downstream concentrations of total dioxins and furans, the congener analysis and the lipid-normalized data suggest that they are not. On a congener basis the data suggest that mussels and SPMDs are more representative of all dioxin and furan exposures. Further, on a lipid-normalized basis there was no statistically significant difference between upstream and downstream locations in the fish data. More importantly, the concentrations were higher upstream than downstream. The caged mussel and SPMD data further suggest that the using the fish test at these upstream and downstream locations is inappropriate since the upstream station appears to be contaminated by another source upstream of the mill. The downstream station was too far away to know whether fish are being exposed to current dioxin and furan discharges from the mill, other sources, or previous discharges from the mill. While the experimental design in the caged mussel pilot study may have been appropriate for comparing dioxin and furan exposures with those in fish and SPMDs, it was not appropriate for addressing the upstream/downstream issues concerning these potential fish surrogates. That would be a gradient design as used in most effluent monitoring studies. Caged mussels and SPMDs should have been placed as close to the pulp mill discharge as possible for a more accurate evaluation of their ability to detect upstream/downstream differences. A more direct approach would be to repeat the caged mussel pilot study with more stations closer to the mill in a gradient design as originally proposed.

With respect to comparing the results of the two surrogate tests evaluated as part of this study, the following conclusions reached by DEP in their 2000 Dioxin Monitoring Report are not scientifically defensible based on the available data. "Since the development of the Above/Below test began in 1997, over 78 tests have been conducted for different dioxins, species, tissue types, and other surrogates in an attempt to develop a test powerful enough to accurately measure any differences above and below a mill. Bass and semi-permeable membrane devices show the most promise and will be tested again in the 2001 program. Freshwater mussels did not appear to be a useful monitoring device, perhaps because they are at a lower trophic level than fish."

There are no data or statements in the DEP report that support these conclusions. The concentrations of dioxins/furans in fish were significantly higher downstream than upstream, the difference was very small and on a lipid-normalized basis the dioxin/furan concentrations were higher in fish upstream than downstream. DEP chose to ignore the lipid-normalized data. Most SPMD samples were below the detection limit, the SPMD data were the most unreliable because reported concentrations were estimated at a fraction of the detection limit, and the response of the mussels was more like fish than the responses of the SPMDs. In addition, approximately 29% and 61% of the total dioxins/furans at upstream and downstream sites was attributable to a single congener (2,3,7,8-tcdf). In almost every study where SPMDs have been compared with mussels, SPMDs have been shown to "over-trap" the lower molecular weight organic compounds. Concentrations of this furan congener were about six times higher than fish or mussels and suggest that the majority of the dioxins/furans from SPMDs did not represent fish or any other living organism and that it was an artifact of the surrogate sampling procedures. In other words the SPMDs were good accumulators of the compound which was least environmentally relevant to fish or mussels. For comparative purposes, 2,3,7,8-tcdf is approximately 1/20 as toxic as 2,3,7,8-tcdd.

This integrated pilot study compared three approaches as alternative monitoring tools for assessing the fate and effects of dioxins and furans associated with a pulp mill effluent. While water samples

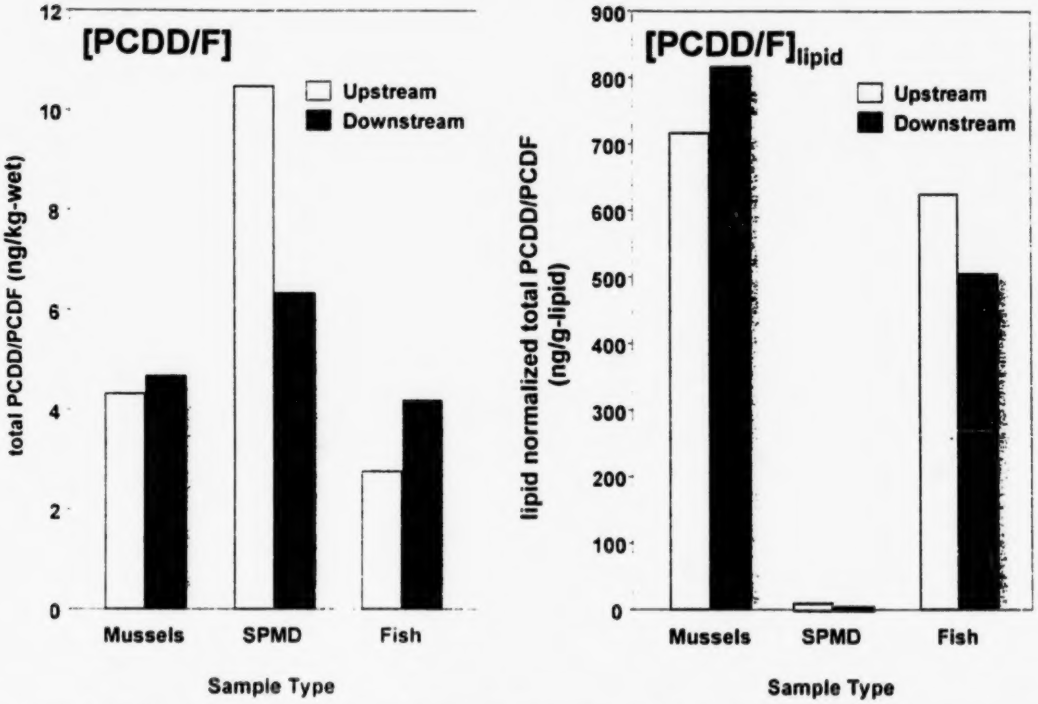


Figure 4. Total dioxins and furans in caged mussel tissues, SPMDs, and fish from upstream and downstream stations, and on a lipid-normalized basis.

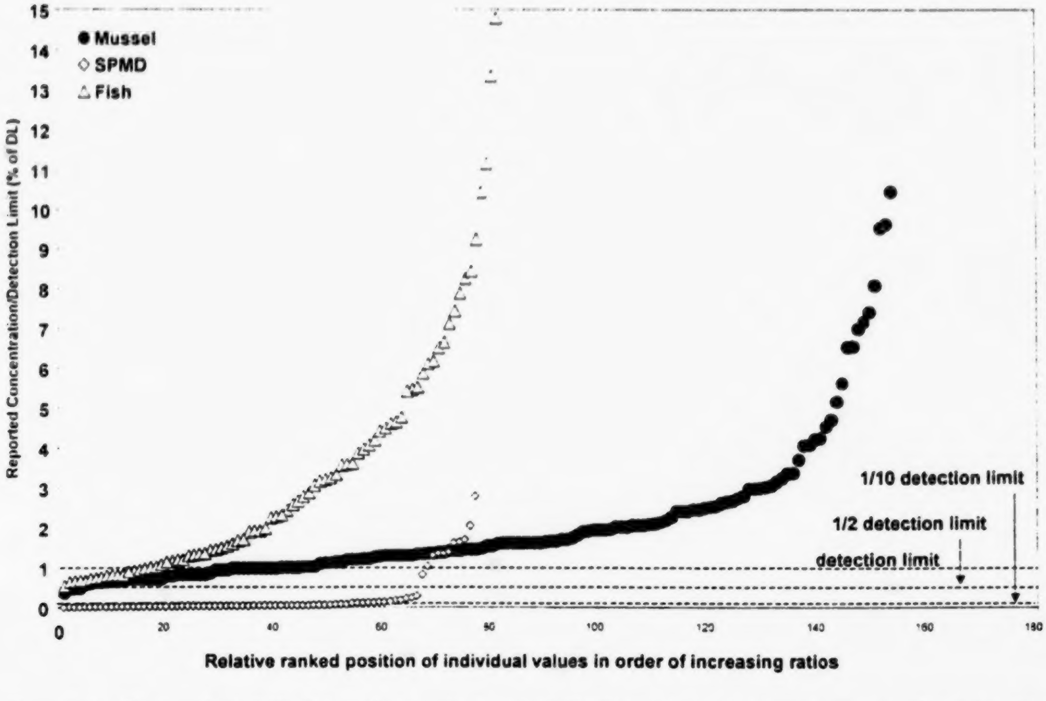


Figure 5. Percent distribution of reported concentrations from congener-specific analyses for mussels, SPMDs, and fish.

have been used to characterize aqueous chemical exposures for over 50 years, new elements used here include the use of caged mussels to integrate chemical exposure and associated biological effects. Caged mussels have been used for approximately 30 years, but recent refinements have increased the sensitivity of this approach to a new level, and these methods have only recently been adopted by the ASTM. SPMDs represent the newest of these methodologies and applications of this approach are still being refined. This study is unique not only in terms of comparing these three monitoring methods, but applying them in areas where they have not been commonly measured in Maine, using state-of-the-art chemical analyses with low detection limits, and using extensive experience and expertise to interpret the results of congener analysis (i.e., dioxins and furans) and mussel growth rates.

There are too many uncertainties in the results from accumulation of dioxins and furans in caged mussels, SPMDs, and fish tissues to unconditionally accept the results and make important programmatic decisions regarding the utility of these three methods. Another pilot study is suggested that directly tests the utility of the caged mussel methodology (and SPMDs) using a gradient design downstream from the mill and placing cages as close as possible to the effluent discharge. The weight of evidence from bivalve biomonitoring studies conducted on chlorinated hydrocarbons such as dioxins furans, and PCBs throughout the world suggest that caged bivalves can be an effective monitoring tool for pulp and paper mill effluents in the State of Maine. This is not to say that bivalves should be the only monitoring tool. Most experts have agreed that there is no perfect monitoring tool and that a weight of evidence approach should be used to make the most meaningful assessments. It seems reasonable to assume that a triad approach using caged mussels, SPMDs, and fish would provide DEP with the best possible data to make informed decisions with respect to potential exposure from dioxins and furans from pulp and paper mills on the Kennebec River. We have previously suggested that the best way to measure water quality is to not measure chemicals in water but measure them in mussel tissues because they provide a more integrated picture of exposure. As anomalous as it may seem, the best way to quantify exposure in fish may be to measure chemicals in caged mussels rather than fish. Mussels are potentially better indicators of dioxin/furan exposures because they do not move and because they can be placed closer to the mill.

Summary

Mussels detected more congeners than either fish or lipid bags. The total concentration of dioxins/furans in mussel tissues were higher downstream than upstream whether or not the data were lipid normalized. More reported concentrations from congener-specific analyses were above the detection limit for mussel tissues than either fish tissues or SPMDs.

Conclusions

Surrogate mussels may be a better indicator of exposure than fish or SPMDs. Surrogate mussels have a greater potential for the above/below test because they don't move and could be transplanted along suspected chemical gradients. DEP was biased in their interpretation of available data.

Recommendations

Conduct another study using gradient design with stations close to mill. Have samples analyzed by another lab to avoid bias and poor methodology. Require documentation from DEP to support conclusions in 2000 report.

Toxicity Evaluation of Sodium Sulphide Using Acute Liquid Phase Microtox® Test. K.J. Kinnee[1], C.V. Eickhoff[1], R. Wood[1], S. Moon[2] and B.K. Firth[2]. [1]BC Research, Inc., Vancouver, BC; and [2]Weyerhaeuser, Federal Way, WA.

A pulp and paper mill was examining toxicity problems using Microtox® tests, and sulphide was suspected as the toxic agent. No literature data concerning the toxicity of sulphide towards marine bacteria was obtainable so BC Research was contracted to assess the effect of sulphide to *Vibrio fischeri*. The acute liquid phase Microtox® basic test was conducted, with some deviations, on salinity (20‰ w/v) and pH-adjusted (7.5), MOPS (3-[N-Morpholino]propanesulfonic acid) buffered sodium sulphide (Na_2S-$9H_2O$) solutions. A total of four Microtox® tests were conducted. For each test, six sulphide concentrations and duplicate negative controls were prepared using buffered pH and salinity adjusted dechlorinated Vancouver city water. Light readings were measured at 5, 15 and 30 minutes of exposure, using a Microtox® 500 analyzer. The pH and salinity of each of the sulphide solutions was measured before and after the tests were completed. Sulphide concentrations in the test solutions were determined iodometrically. The measured concentrations of sulphide in the test solutions were used to calculate the EC50 values. The mean EC50 values, based on reduction in light production, and standard deviations for 5, 15 and 30 minute exposures to MOPS buffered sodium sulphide solutions (pH 7.5) were 91.4±10.6; 57.7±5.5; and 49.5±3.0, respectively.

An Evaluation of the Use of Tracers in Cycle 2 of the Pulp and Paper EEM Program to Determine if Fish were Exposed to Effluent. W.R. Parker and M. Mallory. Environment Canada, Environmental Protection Branch, Fredericton, NB.

The first cycle of the pulp and paper EEM program recommended the use of chemical tracers which were to be measured in fish tissue to confirm that fish captured near pulp and paper mills had been exposed to mill effluent. Following Cycle 1, a technical working group reviewed the use of these tracers and made a series of recommendations about the use of tracers in future EEM cycles. Those recommendations were adopted and were included in the EEM requirements document for Cycle 2 of the pulp and paper EEM program. This poster presents a summary of the national Cycle 2 results on the use of effluent tracers in fish and provides information on the chemical tracers used, the frequency of use and the usefulness of these tracers to confirm that fish were exposed to effluent.

Identification of Toxicity Associated with Lignin Derived Molecules in Pulp and Paper Mill Effluent. C.V. Eickhoff[1], K.J. Kinnee[1], J.S. Pickard[1] and A.H. Kilback[2]. [1]BC Research Inc., Vancouver, BC; and [2]NorskeCanada, Powell River, BC.

A Phase III toxicity identification evaluation (TIE) was performed on effluent samples obtained from a coastal pulp and paper mill in British Columbia. This TIE was conducted to further isolate the components of the effluent which had caused sublethal toxicity in echinoderm (*Strongylocentrotus purpuratus*) fertilization tests. Previous experiments indicated that high molecular weight, non-volatile molecules (HMW) were responsible for the toxicity. Two possible sources of HMW compounds were naturally occurring lignin derived molecules (LDM), from the wood fibre, and amine polymer products, used as sizing agents or flocculants. In this study, the effluent was fractionated into components based on molecular size and polarity to determine the exact cause of the toxicity. Methods used to fractionate the effluent included pH adjustment and solid phase extraction (SPE); ultrafiltration based on molecular weight; and electrophoretic separation of the effluent.

The SPE tests revealed that HMW, non-polar organic compounds that were non-ionised at low pH caused most of the toxicity. The echinoderm toxicity tests performed on the ultrafiltration fractions indicated that HMW molecules >10,000 Da were responsible for the majority of the effluent toxicity. The electrophoretic separation of the HMW effluent components confirmed that the source of the toxicity in the effluent was LDM and most likely not due to the presence of other high molecular weight molecules such as polymers.

The Potential for Using Stable Isotopes to Trace Fish Exposure to Pulp Mill Effluents. M.G. Dubé and L.I. Wassenaar. Environment Canada, National Water Research Institute, Saskatoon, SK.

Our objective was to use ä^{13}C, ä^{15}N, ä^{34}S, ä^{37}Cl to: [1] characterize the isotopic signatures of Canadian pulp mill effluents (PMEs), and [2] assess fish tissue assimilation of stable isotopes in a laboratory exposure. PMEs were collected from a cross section of process types and treatment strategies. Analyses of the effluents revealed signature ranges of –25.5 to –14.7 for ä^{13}C, -4.0 to –2.6 for ä^{15}N, -3.7 to 16.5 for ä^{34}S, and –1.7 to 0.8 for ä^{37}Cl. Principal components analyses showed that each PME had a distinct isotopic "fingerprint." Juvenile rainbow trout (*Onchorhynchus mykiss*) were raised for 45 days in the laboratory under control and 10% PME treatments. Fish within each treatment were fed chironomids (*Chironomus tentans*) cultured under either control conditions or under effluent exposed conditions (10%).

There were no significant differences among treatments with respect to ä^{13}C, ä^{15}N, or ä^{34}S. PME was significantly depleted in ä^{37}Cl (-1.690) compared to the laboratory dilution water (0.612). Chironomids exposed to 10% PME were significantly depleted in ä^{37}Cl (-2.34) compared to controls (-0.792). Trout exposed to 10% PME were significantly depleted in ä^{37}Cl relative to controls and the depletion resulted from waterborne exposure rather than through their food source. These results are significant because they suggest that ä^{37}Cl may be a tracer of biotic exposure to PME.

Use of Static Renewal and Flow-Through Tests to Assess the Estrogenicity of Effluent from a Thermomechanical Pulp and Paper Mill in Ontario. J.P. Sherry[1], C. Tinson[1], J.L. Parrott[1], D. Trowbridge[2], S. Verma[2], S. Cuddy[3] and K. Cooper[1]. [1]Environment Canada, National Water Research Institute, Burlington, ON; [2]Sault College, Sault St. Marie, ON; and [3]ULERN, Sault St. Marie, ON.

Results from *in vitro* tests have shown that effluents from thermomechanical pulp mills (TMP) contain estrogenic chemicals, and thus have the potential to cause estrogenic responses in fish. Our objective was to test the ability of effluent from a Northern Ontario TMP mill to induce vitellogenin (Vg) in juvenile rainbow trout. We used a static renewal procedure to test the potency effluent in the laboratory. The effluent in the exposure chambers was fully renewed daily for 21 days. A flow-through regime was used to test the ability of an environmentally relevant dilution of the effluent (2%) to induce Vg in juvenile trout over a two month exposure. For both experiments, a replicated tank design was used to allow for statistical testing of tank effects. The data will show whether final effluent from this TMP mill can induce Vg in rainbow trout. In addition we shall compare the *in vivo* results to those from an *in vitro* test of the effluent's estrogenic potential in primary cultures of rainbow trout hepatocytes.

Do Pulp Mill Biosolids Pose a Toxicity Hazard to Fish? S.A. Hawkins[1], C. Hedley[1], H. Orr[1], M.R. van den Heuvel[2] and P.V. Hodson[1]. [1]School of Environmental Studies, Queen's University, Kingston, ON; and [2]Forest Research, Rotorua, New Zealand.

The use of pulp mill biosolids as a soil amendment is an increasingly prevalent practice. The potential risks of biosolids to aquatic biota may vary with treatment process, wood furnish, and phase of treatment. We sampled final combined biosolids from thermomechanical (TMP) and bleached kraft (BKM) pulp mills using activated sludge treatment systems, and separate primary and secondary biosolids from an aerated stabilization basin system treating the combined effluent of TMP and BKM mills. Relationships between toxicity, BOD, TOC, and the bioavailability and potency of toxic constituents were examined at acute and sublethal biosolid concentrations. CYP1A induction was observed only in the TMP/BKM secondary samples obtained from the stabilization ponds. These were the only samples with appreciable concentrations retene, the product of anaerobic abietic acid degradation. These results suggest that more biotransformation processes may occur in static treatment systems with longer retention times than in those with continual turnover. Chronic testing with early life stages of trout suggested that biosolid toxicity was due to chemical as opposed to physical interactions, and that the toxic constituents were at least moderately water soluble. Our findings suggest that effluent constituents are not entirely mineralized during treatment, but persist or are biotransformed and continue to pose a toxicity hazard to aquatic species.

Cardiac Output, Swimming Performance, and Haematological Effects of Acute Exposure to Thermomechanical Pulp Mill Effluent on Prespawning Adult Atlantic Salmon (*Salmo salar*). E.D. Linton[1], R.S. McKinley[1] and D.A. Scruton[2]. [1]Centre for Aquaculture and the Environment, University of British Columbia, Vancouver, BC; and [2]Department of Fisheries and Oceans, St. John's, NF.

Atlantic salmon (*Salmo salar*) is a culturally and recreationally important species in the Exploits River, Newfoundland. Salmon angling in this river, which is the longest and boasts the largest drainage basin on the island, rivals that found worldwide. As this river is so vast, it also coexists with urban centres, industries, and a golf course that lie adjacent to its banks. The principal point source of xenobiotic input to the river originates from a thermomechanical (TMP) newsprint mill. Salmon returning to the river to spawn encounter low concentrations of effluent as they move inland.

The effects of an acute, 6 hour exposure to 6, 12, and 25% (v/v) TMP effluent on cardiac output, swimming performance, and blood parameters including haematocrit, glucose, and osmolality were examined in wild adult Atlantic salmon under laboratory conditions. Cardiac output and haematological parameters were also examined while fish were also exposed to a gradually increasing concentration gradient of effluent (0 to 25% v/v), mimicking upstream movement past the outfall. No significant changes were observed in any of the endpoints examined, thus indicating that the pulp and paper mill in question is not impairing the spawning migration or acting as an acute stressor to the fish. The potential for using cardiac output as a biomonitoring tool of environmental change is also discussed.

Metal Mining Environmental Effects Monitoring

Session Co-chairs: K. Liber and L. Trudel

New Federal Metal Mining Effluent Regulations - Implementation of the Environmental Effects Monitoring Program. L. Trudel, K. Hedley and S.C. Ribey. Environment Canada, National EEM Office, Hull, QC.

The new *Metal Mining Effluent Regulations* (MMER) under the *Fisheries Act* were registered and published in the Canada Gazette Part II in June 2002. All mines regulated under the MMER are required to conduct environmental effect monitoring (EEM) as part of their authority to deposit effluent. The EEM program requires mines to monitor fish, benthic invertebrates, water, sediment, effluent and sublethal toxicity to determine if the mining effluent is having an impact on the aquatic environment. The main elements of the EEM program are a fish population survey, a benthic invertebrate community survey and a fish tissue analysis to evaluate effects on fish, fish habitat and the usability of fisheries resources. The program will be implemented similar to the Pulp and Paper EEM program. Mines will be required to submit to Environment Canada study designs prior to proposed field work, and interpretive reports after completion of field work. Environment Canada, through the Regional Offices, will review proposed study designs and interpretive reports. This talk will focus on the requirements of the EEM program and will include the timetable for conducting monitoring and for submission of study designs and interpretative reports. Technical changes incorporated to the requirements after Gazette 1 public consultation will be also presented.

Preparing for the Mining Environmental Effects Monitoring Program by Assessing Metal Bioavailability in Effluents. B. Vigneault and R. Prairie. Noranda Inc., Technology Centre, Montréal, QC.

Canadian mines will be subjected to revised federal effluent regulation, MMER, promulgated in June 2002, which include a requirement to conduct an Environmental Effects Monitoring (EEM) program. The results of 2 year project at Noranda on metal bioavailability and toxicity in effluents will provide targeted mining sites relevant information for the interpretation of the data collected in the revised MMER. Our results indicate that total metal concentrations are poor predictors of effluent toxicity and that, for some toxicity tests, major cations and not trace metals, are responsible for the observed toxicity. Complexation of free metal ion is important in effluents particularly for Cu. Our project results will also help the mine sites to prepare their first EEM study design, due in less than a year, and for the interpretation of the subsequent monitoring and toxicity data that will be collected. The project characterised the chemistry and also help to identify the chronic toxicity tests that will be problematic and to identify the cause of the observed toxicity. Such information will be crucial for the interpretative report and for the investigation of cause. Also, these results will be used to demonstrate the importance of trace metal speciation within the context of the new Quebec regulation including environmental discharge objectives.

Effluent Toxicity Evaluation of the Teck Cominco, Trail Operations Smelter Using a Revised Model (1995 to 2000). W.F. Duncan. Teck Cominco Metals Ltd., Trail, BC.

Teck Cominco has been working to protect the environment by reducing the metal loads to the Columbia River and ultimately, the toxicity of its effluent discharges. The new KIVCET lead smelter has significantly reduced emissions and effluent discharges, assisting Trail Operations in reducing metal releases to the environment. KIVCET's startup in 1997 essentially concluded the original Trail Modernization Program, which was begun in 1977. When the KIVCET Lead Smelter began running at greater than 90% of capacity during December of 1998, this triggered the beginning of a 12 month environmental performance evaluation as required under Permit. A review of the chemical and biological assays conducted on three effluent streams and Stoney Creek will be presented. In evaluating the data, we make use of the Toxic Unit Model concept (Duncan and Antcliffe, 1996), a useful evaluation and planning tool designed to help anticipate bioassay results based on water chemistry results. This model has proven very useful in evaluating the effluent streams and in indicating the elements creating toxicity in those streams. The model has been revised based on the additional data collected. The model assists in assuring non-acute toxicity at discharge points into receiving waters. Toxicity reduction goals have been largely met over the course of the Trail Modernization Program. The focus is now shifting towards the Environmental Management System (EMS), which emphasizes small continual improvements to effluent streams.

Development of an Environmental Effects Monitoring Program for the Eskay Creek Mine. H.C. Bailey[1], E.J. Raggett[1], P.M. Chapman[1] and F.M. Murphy[2]. [1]EVS Environment Consultants, North Vancouver, BC; and [2]Homestake Canada, Smithers, BC.

Environmental Effects Monitoring (EEM) is an important component of metal mining operations which is used to determine if mine discharges are causing adverse effects to the aquatic receiving environment. In 1997, prior to the publication of Environment Canada's draft EEM guidance document, Homestake Canada, Inc. requested that EVS Environment Consultants implement an EEM Program for the Eskay Creek Mine. Mining operations at this site result in the production of mine water, waste rock and tailings, which Homestake is authorized to discharge. The Eskay Creek Mine EEM program was designed to assess whether mine discharges cause exceedances of the BC Freshwater Aquatic Life Criteria in the receiving environment and/or adverse effects to resident aquatic biota. The program includes monitoring of water, sediment, periphyton, benthic invertebrate communities, and bioaccumulation studies (no fish are present in the creeks draining the mine site). Results of the EEM studies show that water and sediment chemistry appear to be minimally impacted by mine discharges, with several criteria exceedances due to naturally high metal concentrations from the mineral rich drainage. Elevated sediment metal concentrations are present in Ketchum Creek downstream of mine discharges. However, the benthic invertebrate community increases in both abundance and number of taxa, which suggests that metals are not adversely affecting the community. Overall, discharges from the mine do not appear to be impacting sediment or water quality in the Unuk River as this system contains naturally high metals concentrations due to the mineralized drainage. Following the conclusion of each annual EEM program, results are reviewed to ensure that the objectives of the program are being met. This program is an excellent example of how science and adaptive management can be used to help mines achieve their environmental monitoring goals.

Using Mesocosms to Assess Metal Mine Effluent (MME) Effects on Fish and a Benthic Invertebrate, Junction Creek, ON. K.A. Hruska[1], M.G. Dubé[2], G.D. Watson[3] and D.L. MacLatchy[4]. [1]Toxicology Centre and National Water Research Institute, University of Saskatchewan, Saskatoon, SK; [2]Environment Canada, National Water Research Institute, Saskatoon, SK; [3]INCO Ltd., Copper Cliff, ON; and [4]Department of Biology, University of New Brunswick, Saint John, NB.

In 2001 and 2002, we furthered development of our mesocosm technology for conducting metal mining EEM assessments for fish [creek chub (*Semotilus atromaculatus*); northern redbelly dace (*Phoxinus eos*)] and a benthic invertebrate (*Chirnomus tentans*). The INCO site is highly confounded with three treated mining effluents discharged into Junction Creek (Garson Mine, Nolin Creek Waste Water Treatment Plant (NCWWTP), and Copper Cliff Waste Water Treatment Plant (CCWWTP)). In 2001, creek chub were exposed for 35 d to: [1] control water, [2] Garson effluent (30%), [3] NCWWTP effluent (20%), [4] CCWWTP effluent (45%), and [5] Garson (2%) + NCWWTP (10%) + CCWWTP (45%) effluent. Effluent exposure resulted in significant reductions in the survival of adult and juvenile creek chub. Gonad size was reduced in males exposed to all effluent treatments compared to controls. Production of plasma testosterone in both sexes was significantly reduced by exposure to MME. Tissue metal levels were significantly increased in effluent-exposed fish. In 2002, follow-up studies were conducted. The effects of CCWWTP, NCWWTP and Garson mine effluent on creek chub and northern redbelly dace were examined. In addition, effects of CCWWTP effluent on the lifecycle of *Chironomus tentans* was examined using a mesocosm system.

Whether 'Tis Better to Grab or Kick in Enviromental Effects Monitoring Benthic Invertebrate Assessments. D.T. Zaranko[1], P. Orr[2], D. Farara[3], I. Martin[4] and C.D. Wren[5]. [1]Zaranko Environmental Assessment Services, Guelph, ON; [2]Minnow Environmental Inc., Mississauga, ON; [3]Beak International Inc., Brampton, ON; [4]Ian Martin Biological Consulting, Elora, ON; and [5]ESG International, Guelph, ON.

Traditional benthic invertebrate assessments, such as those widely practised in Cycles 1 and 2 Environmental Effects Monitoring (EEM) programs at pulp and paper mills, employ quantitative, unit-area sampling. Taxonomic identification has been done to the lowest practical level, often genus or species. Alternative, rapid assessment approaches for benthic community monitoring have become widely used in the U.S., U.K., and Australia (Taylor, 1997; Reynoldson et al., 1995). These typically employ a single pooled sample collected from each sampling area with a standard effort (time), using a D-frame or other kick-net (Taylor, 1997). The entire sample or some fixed proportion of it (e.g., first 100, 200 or 300 animals) is sorted and identified, often only to family.

The advantage of this type of approach is that it requires minimal expertise and equipment. There is a perception, too, that such studies are less expensive, although actual cost savings may be minimal. The option to collect standard-effort (timed) kick samples rather than area-delimited samples is provided in the latest EEM guidance for pulp and paper mills (p. 6-42 and 6-43 in Environment Canada, 1998) and is specifically recommended in the context of the Reference Condition Approach in the draft guidance for metal mines (p. 5-90 to 5-92). This presentation will evaluate features of the rapid assessment approach for comparison to the quantitative sampling approach typically employed in regulatory programs, including EEM studies, during the past decade. The presentation will discuss technical considerations, cost, and the types of questions being addressed in environmental monitoring programs. The objective will be to identify the circumstances under which each approach is most appropriate.

Using Bray-Curtis Distances in Environmental Effects Monitoring Invertebrate Community Surveys. M.D. Paine. Paine, Ledge and Associates (PLA), North Vancouver, BC.

In the metal mine Environmental Effects Monitoring, Invertebrate Community Survey, Bray- Curtis (B-C) distances (*D*) are used to assess effects on invertebrate community composition. *D* provides a measure of the degree of non-overlap, or the number or proportion of organisms not shared, between samples. The EEM guidance document recommends using raw abundances to calculate distances between reference or exposed samples, and reference medians. If raw abundances are used, distances and community differences among samples or between areas will be correlated with differences in total abundances (*N*) (i.e., *D* and *N* will be partly redundant). That redundancy can be reduced or removed by using relative abundances, or taxon abundances as a percentage of the total. *D* will then be percent difference, a comprehensible and widely recommended measure of differences in community composition, fish diets, and ecological niches. Other transformations are statistically unnecessary, inflate the importance of rare species, and reduce the comprehensibility of *D* values. If distances are calculated between samples and some common or fixed "standard", such as reference medians, those distances can be compared among areas in *t* tests, ANOVA or non-parametric equivalents. That approach provides a single independent measure or value per sample, but results will depend on the choice of standard. A better approach is to calculate average pair-wise distances between versus within areas, and compare that value to values obtained by randomly re-sampling the data (=bootstrapping).

Potential Uses and Misuses of Ecological (Dis)similarity Measures in Environmental Monitoring. B.W. Kilgour[1], K.M. Somers[2] and D.R. Barton[3]. [1]Jacques Whitford Environment Ltd., Ottawa, ON; [2]Ontario Ministry of the Environment and Energy, Dorset, ON; and [3]Department of Biology, University of Waterloo, Waterloo, ON.

This study examined the relative sensitivities of seven commonly used indices of stream benthic community composition and three multivariate indices to effects associated with mines, pulp and paper mills, and urbanization. The indices included: total abundance, number of taxa, diversity (H'), evenness (J'), Hilsenhoff's Biotic Index (HBI), the BioMAP water quality index (WQI), the percent model affinity (PMA), and the first three ordination axes from a correspondence analysis (CA). Six data sets (i.e., two from each of the three types of development) were used to address the objectives. In each data set, replicate benthic samples were collected from reference areas as well as one or more downstream areas exposed to a point-source or non-point source discharge.

The PMA approach and the ordination axes indicated significant differences between the reference and downstream communities for all six data sets ($p<0.05$). With the exception of H' diversity, each of the other metrics revealed significant effects associated with one or two, but not all three types of development. For example, the HBI and WQI indicated significant effects associated with pulp and paper and urbanization, but not mining. In all studies, effect sizes (i.e., the standardized difference between means for the reference and exposed areas expressed in units of standard deviations (SDs) exceeded 2 SDs. However, effect sizes for the PMA and the first or second ordination axis scores were generally larger than effect sizes for the other metrics indicating that these indices were often the most sensitive indicators of development. In addition, there was a high degree of redundancy among the various metrics, perhaps because the effects were substantial in most of the data sets. The greater sensitivity of the ordination axes and the PMA approach emphasized their value as indices of benthic community composition. As a result, we recommend that any suite of indices used for assessing benthic communities should include these types of multivariate metrics.

Use of Stream Mesocosms to Assess Metal Mining Discharge Scenarios: Direct and Indirect Effects on Benthic Food Webs. J.M. Culp[1,2], N.E. Glozier[1], K.J. Cash[1], M.G. Dubé[1], D.L.

MacLatchy[2], R. Prairie[3] and C.S. Wood[3]. [1]Environment Canada, National Water Research Institute, Saskatoon, SK; [2]Departments of Biology, University of New Brunswick, Fredericton and Saint John, NB; and [3]Noranda Inc., Centre de Technologie, Pointe-Claire, QC.

Mesocosms can of integrated monitoring strategies for riverine food webs, especially when complex exposure dynamics exist or when future scenarios need evaluation. We used mesocosms to assess the effects of 0%, 20% and 80% (v/v) of a metal mining effluent (MME) on the benthic food web (algae to fish) of the Little River near Bathurst, NB. Treatment levels included existing MME discharge (80%) and the predicted discharge scenario upon mine closure (20%). Measurement endpoints were algal biomass, invertebrate community composition, insect emergence, and slimy sculpin survival and growth. Invertebrate community composition, richness and evenness were strongly affected by 80% MME. Insect emergence from 0% and 20% mesocosms was similar; however, few insects emerged from the 80% MME treatment. In contrast, algal biomass increased sharply in 80% MME, apparently in response to grazing pressure release. Over the 26 day experiment, sculpins exposed to 0% or 20% MME were larger than individuals exposed to 80% MME; only 25% of the 80% MME fish survived. The mesocosm approach produced consistent results among different levels of biological organization and provided important predictions for environmental effects assessment of present and future MME discharge scenarios.

Use of Toxicity Identification Evaluation Procedures as Part of an Environmental Effects Monitoring Program for a Mine. J.R. Elphick[1], H.C. Bailey[1] and F.M. Murphy[2]. [1]EVS Environment Consultants, North Vancouver, BC; and [2]Homestake Canada, Smithers, BC.

The toxicity of discharges from mining operations continue to be of concern to the regulatory community and mine operators. Toxicity in discharges may be caused by a variety of factors, including metals, ammonia, pH, process chemicals and total dissolved solids. In this study, a toxicity identification evaluation (TIE) was performed on samples of a discharge from a gold and silver mine which exhibited toxicity in 7 day partial lifecycle tests using the freshwater cladoceran *Ceriodaphnia dubia*. The results indicated that an organic anion was responsible for toxicity and that phosphorus concentrations in the treatments were correlated with toxicity. Collectively, the data suggested that a phosphine-based collector (Aerophine 3418A Promoter) used in the metals flotation process was the most likely cause of the observed toxicity. Consequently, the chemical was evaluated for toxicity and its response to the TIE procedures which were effective at reducing toxicity in the discharge sample. These results, and those of a confirmatory spiking study, consistently suggested that Aerophine was the cause toxicity. Efforts at the mine to reduce the residual concentration of this process chemical resulted in reduced toxicity of the discharge.

Contributions of Sediment Geochemical Fractions to the Metal Body Burdens of Benthic Invertebrates. J.C. Evans and D.G. Dixon. Department of Biology, University of Waterloo, Waterloo. ON.

The determination of metal contributions from the solid phases of sediment is often confounded by the presence of highly bioavailable metal fractions in the overlying and pore-water compartments. This problem can be exacerbated by traditional sediment spiking techniques where metals are added to sediment as dissolved salts, which can readily repartition into the overlying water. Our study used a recirculating tank design in which a single pool of water is aerated and forced through multiple sediment test containers; a system which eliminates differences between the overlying waters in the test containers and allows for a large (50:1 total) water to sediment ratio. This system also increased the exposure of the test sediment to the oxic overlying water pool, an important consideration in that the trace metal spikes were added as iron oxyhydroxide co-precipitates. Benthic invertebrates (*Tubifex tubifex*, *Chironomus* spp. and *Hyalella azteca*) exposed to various solid-phase concentrations of metal-spiked sediments (Cd, Cu and Ni, singly or in combination), mine tailings or sediments from mining-impacted lakes for 3 to 5 weeks were gut-purged in fresh sediment and their tissue metal contents determined. Results were used to build a preliminary model of sediment metal bioavailability. Geochemical fractionation of the sediment metals after the test period revealed that the metal spikes did not remain exclusively in the iron-bound phase - extensive repartitioning took place during the experiments, especially into the organic phase of the sediment.

Influence of Water Chemistry on the Chronic Toxicity of Copper in *Ceriodaphnia dubia*. M.L. Schwartz and J.C. McGeer. Natural Resources Canada, Mining and Mineral Sciences Laboratories-CANMET, Ottawa, ON.

The development of biotic ligand modeling approaches has dramatically improved the understanding and prediction of the toxicity of metals in fresh waters. While biotic ligand models (BLMs) are being successfully developed for acute toxicity, their extension to chronic toxicity has been more challenging. The objective of our studies was to develop a chronic biotic ligand model for waterborne Cu in the invertebrate *Ceriodaphnia dubia* and provide data towards site specific chronic toxicity predictions. Using the standard seven day survival and reproduction test, the presence of competing cations such as Ca^{2+} and Na^{+}, as well as complexing anions including natural organic matter on toxicological responses in moderately soft water were evaluated. For example Ca^{2+}, up to concentrations of 20 mmol/L (800 mg/L), did not alter the impact of Cu on survival or reproduction in *C. dubia* although Ca^{2+} itself did produce negative effects at levels above 12.5 mmol/L (500 mg/L). The toxicological endpoints were correlated with water chemistry using the geochemical speciation software MINEQL+. The results of the research are directed at improving regulatory approaches for metals in aquatic systems including water quality guidelines and criteria as well as hazard and risk assessment methodologies.

Effects of Combinations of Zinc, Cadmium and Aluminum, on the Survival of *Ceriodaphnia dubia*. B.L. Rudolph, J. Young, J.R. Elphick and H.C. Bailey. EVS Environment Consultants, North Vancouver, BC.

A study was conducted to evaluate the toxicity of selected metals when present in combination on the survival of *Ceriodaphnia dubia* at two water hardnesses. The metals used for this investigation were Zn, Cd and Al. The 48 hour LC50 values were determined for each metal alone and in combination with each of the other metals as well as for a combination of all three metals. The results demonstrated a difference in LC50 values based on water hardness; toxicity was greater at lower hardness. In combining the metals, it was observed that toxicity of the metals were largely additive, or slightly less than additive, regardless of water hardness.

Toxicity Identification Evaluation for Stormwater Runoff from an Aluminum Smelter. J.R. Elphick and H.C. Bailey. EVS Environment Consultants, North Vancouver, BC.

A toxicity identification evaluation (TIE) was performed on samples of stormwater runoff from an Al smelter which exhibited toxicity in larval development tests with the marine mussel *Mytilus galloprovincialis*. The observed pattern of toxicity was unusual because of a flat and sometimes inverted dose-response. The results of the TIE indicated that Al was responsible for toxicity, with the dose-response a function of the low solubility of Al and the interaction between Al, fluoride and pH. Supportive data included toxicity tests with Al at different pH values and concentrations of fluoride. Fluoride addition resulted in a large decrease in toxicity of Al; however, pH adjustment did not alter the toxicity of Al tested without fluoride. As part of the TIE, the cause of toxicity to was also evaluated with the freshwater cladoceran, *Ceriodaphnia dubia,* and was also attributed to Al.

Fitness Indicators of Benthic Invertebrates as an Assessment Tool in Environmental Effects Monitoring. N. Munteanu and G.P. Thomas. G3 Consulting Ltd., Burnaby, BC.

Benthic invertebrates have been useful in environmental monitoring assessments such as EEM and ecological risk assessment, given their ubiquity, sedentary behaviour, ease of collection, and demonstrated tolerances and intolerances to specific contaminants. Use of indigenous benthic communities was successful in demonstrating extent and magnitude of exposure to specific perturbations in EEM investigations throughout Canada. This paper explores the utility in EEM of population-level endpoints, "fitness parameters", used in mining investigations throughout Canada. Fitness parameters may also assist in further defining the effects of discharges to receiving environment communities. Use of fitness parameters would provide elucidation and differentiation from mill-effect of system-associated effects previously too subtle to distinguish at the community level. Examples of fitness parameters at the individual/population level include size, condition, and fluctuating asymmetry. Common deformities include numerical asymmetry, bilateral asymmetry and reversion. The merits (and limitations) of utilizing fitness parameters to support community structure assessments (e.g., biomass, species abundance and richness) for EEM assessments is discussed. Such assessments may be particularly useful in studies hampered by confounding influences and other questions of complexity. Additional strengths and weaknesses of the technique are also reviewed in the paper.

Determining the Metal Content of Micro- and Nano-Size Environmental Samples by In-Torch Vaporization-Inductively Coupled Plasma Spectrometry. J.C. Evans[1], H.R. Badiei[2], A.T. Smith[2] and V. Karanassios[2]. [1]Department of Biology, and [2]Department of Chemistry, University of Waterloo, Waterloo, ON.

Tissue body burdens are increasingly in use for evaluating the impact of metals on the environment and as a means of estimating the biological availability of metals in both lab and field samples. Unfortunately, the determination of metals in tissues often requires considerable pre-treatment prior to analysis, usually in the form of digestions which can take several days to complete, use extremely hazardous chemicals, and increase the risk of sample contamination and loss. In addition, the digestate volumes and concentrations required for multi-element analysis by traditional ICP-AES (ICP-Atomic Emission Spectrometry) are sufficiently great that many small or micro-sized samples cannot be analyzed by this method and thus require multiple runs of matrix-sensitive GFAAS (Graphite Furnace Atomic Absorption Spectrometry).

The ITV system was designed as an add-on to commercially available ICP-AES instruments and provides a means for performing simultaneous multi-element analysis of small to micro-sized solid or liquid samples with minimal preparation and detection limits comparable to, and in some cases, lower than those of GFAAS. No digestion is required, even for such refractory materials as sediment-ashing and even drying of fresh samples can all be performed inside the ITV vaporization chamber. Examples presented will include the determination of Pb in micro-sized parings of human fingernails, Zn and Cd simultaneously in neonate *Hyalella azteca*, Ca and Mg in single grains of pollen and Ca in a single cell of *Paramecium* sp.

Accumulation of Metals and Biochemical Changes in Small-Bodied Fish Caged in Gold Mining Effluents. C. Doebel[1], C.L. Baron[2], K.G. Wautier[2], R.E. Evans[2] and V.P. Palace[2]. [1]Environmental Science Program, University of Manitoba, Winnipeg, MB; and [2]Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, MB.

Environmental effects monitoring (EEM) in Canadian metal mines was initiated in 1993 to assess the health of fish and their habitats impacted by mining effluents. Caging fish directly in receiving waters is a useful method for determining effects. However, at this point caging studies are not a recognized methodology in the EEM program. To examine the utility of this approach, Pearl Dace (*Semotilus margarita*) were held in a freshwater system of northern Ontario that receives effluents from two gold mines. Fish were caged: [1] several meters downstream of the tailings pond release point, [2] downstream of the tailings pond, the municipal waste disposal site, and sewage effluent release point and, [3] at two upstream reference sites. After 14 days the fish were frozen whole on dry ice. Whole body homogenates of each fish were analyzed for As, Se, Cd, Cu, Zn, Pb, Ni. Enrichments of several metals were identified in fish exposed to mine effluents versus fish caged at reference sites. Metallothionein was analyzed as an indicator of exposure to metals. Ratios of triiodothyronine:thyroxine were measured as a general indicator of chronic stress. This study illustrates that caged, small-bodied fish can be used to examine accumulation of metals and biochemical effects in mining receiving waters.

Cleavage of Selenomethionine by Fish Embryos Can Generate Oxidative Stress. V.P. Palace [1], J. Spallholz[2], J. Holm[3], K.G. Wautier[1], R.E. Evans[1] and C.L. Baron[1]. [1]Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, MB ;[2]Texas Tech University and Selenium Technologies Inc., Lubbock, TX; and [3]Department of Zoology, University of Manitoba, Winnipeg, MB.

Selenium is an essential component of the vertebrate diet. Despite this requirement, the offspring of oviparous vertebrates exposed to levels of Se only several fold greater than those required, can exhibit toxicity. Toxicity of Se has been attributed to its ability to substitute for sulfur during the assembly of proteins. However, not all of the toxic effects of Se can be explained by this mechanism. Specifically, oxidative stress resulting from redox cycling of some forms of Se has been described.

Selenomethionine, the predominant form of Se in fish eggs, is not capable of redox cycling, but lesions consistent wit oxidative damage have been identified in embryos with high concentrations of Se. Results from a series of experiments will be presented that demonstrate the ability of salmonid embryos, at various stages of development, to transform selenomethionine to forms that are capable of producing superoxide radical. This activity is dependent on the presence of glutathione and can be abolished by the addition of the enzyme superoxide dismutase (SOD). Isolation of this activity from the eggs of trout provides important information regarding the mechanism of toxicity in fish exposed to elevated Se concentrations.

Aquaculture in Open Pits and Tailing Ponds after Mine Closure: A Challenge to Control Acid Mine Drainage. F.A. Otchere[1], M.M. Veiga[1], J.J. Hinton[1] and B. Hamaguchi[2]. [1]Department of Mining and Mineral Process Engineering, University of British Columbia, Vancouver, BC; and [2]Highland Valley Copper, Logan Lake, BC.

Acid rock drainage (ARD) has been described as the largest environmental liability facing mining industry. Of the 16 metals mines currently operating in British Columbia, six are producing ARD and several more have the potential to do so. As flooding of open pits after mining operations is a widely recommended measure for metal mines to inhibit the generation of ARD. Developing aquaculture within flooded open pits and tailings impoundments is in line with government policies for reclamation, restoration and sustainable development required of mining companies. In addition, aquaculture in a controlled environment may be more acceptable to critics of fish farming who are concerned about fish escapes and viral transmissions to wild populations. In keeping with the principles of sustainable development and innovative end land uses, the potential for the development of aquaculture in flooded open pits and tailings impoundments could be a significant contribution to the social equity, economic vitality and environmental responsibility in mining communities. Aquaculture may provide an important long-term economic activity for inhabitants of communities around inactive mines. The main objective of this is to demonstrate that deactivated mines can be used as commercial, recreational or ornamental fish farming ponds and lakes as an effective means to prevent and mitigate the socio-economic conditions left to the surrounding communities. Metal concentrations found in rainbow trout from Highland Valley Copper mine are presented as a study case to show low health risk factor involved even though it is not an example of commercial fish farming venture.

Municipal Waste Water Effluent Effects

Session Co-chairs: J.D. Clarke and B. Munson

Environmental Factors Controlling the Transport, Speciation and Bioavailability of Metals Released from Municipal Wastewater Effluents. C. Gagnon[1], I. Saulnier[1], P. Turcotte[1], F. Gagné[1], C. Blaise[1] and M.H. Salazar[2]. [1]Environment Canada, Centre Saint-Laurent, Montreal, QC; and [2]Applied Biomonitoring, Kirkland, WA.

Environmental impacts of urban wastewater discharge on receiving waters are numerous and inputs of contaminants such as metals can cause toxicity to organisms in receiving waters. Physico-chemical conditions of the receiving waters influence metal speciation and bioavailability. The bioavailability and dietary uptake pathways of those released metals in the receiving waters were investigated following two approaches; chemical determinations of metals in waters, and an exposure experiment with caged mussels. The wastewater effluent generated by the treatment plant of the city of Montreal (Québec), which is the largest one in the St. Lawrence Valley, was investigated under this study. Total and extractable particulate as well as dissolved metal concentrations were determined in surface waters of this effluent dispersion plume. Metal bioaccumulation was investigated in tissues of the mussel specie *Elliptio complanata,* which was exposed to the municipal effluent plume for 90 days. Tissue distributions of certain metals (e.g., Ag, Cd, Cr) provide good tools to distinguish the exposure routes (dissolved vs particulate phase) for mussels exposed to municipal effluents. Results of metal bioaccumulation in mussels showed that metals are generally less bioavailable in the effluent dispersion plume than at the reference site in the Saint-Lawrence river, and that physico-chemical conditions control the metal uptake in the receiving waters.

Measuring Ecdygenic Effects of Municipal Wastewaters to the Brine Shrimp *Artemia franciscana*: A New Type of Endocrine Disruption. F. Gagné and C. Blaise. Environment Canada, Centre Saint-Laurent, Montréal, QC.

Municipal effluents are known to release several classes of chemicals that can perturb the endocrine system of aquatic oviparous organisms. The objectives of this study were twofold: [1] to find biochemical endpoints related to the molting hormone ecdysone of the brine shrimp *Artemia franciscana*, and [2] to explore the ecdygenic properties of municipal wastewaters. A novel assay is proposed to detect steroid-induced protein binding to genomic DNA. Shrimps were exposed to increasing concentrations of 11-hydroxyecdysone and municipal effluent extracts (MEE) for 48 hours at 20°C. Then, the levels of vitellogenin (via alkali-labile phosphates, or ALP, in proteins), acetylcholinesterase and activation of DNA-protein interaction were measured in exposed animals.

The results showed that both ecdysone and the MEE increased the levels of ALP in proteins at a threshold concentration of 0.03 µM for the former. Increased protein ALP occurred with the appearance of a very high molecular weight protein complex displaying similarity to vitellogenin. Acetylcholinesterase activity was also induced by ecdysone and the MEE indicating increased catabolism of the neurotransmitter acetylcholine. Protein-binding to genomic DNA in the brine shrimp indicates that both ecdysone and the MEE increased the binding of protein to DNA as determined by acid precipitation. E2 had little effect while nonylphenol, a municipal surfactant biodegradation product, increased protein-binding to DNA suggesting that these chemicals are bioactive with the following ranking potency: ecdysone > nonylphenol > E2. Overall, results suggest that the biomarkers examined in this study are responsive to ecdysone and that a primary-treated municipal effluent contains ecdygenic compounds.

Long-Term Exposure of Fresh-Water Mussels to a Municipal Effluent Plume Increases the Number of Females. F. Gagné[1], C. Blaise[1], M. Douville[1], S. Trottier[1] and M.H. Salazar[2]. [1]Environment Canada, Centre Saint-Laurent, Montreal, QC; and [2]Applied Biomonitoring, Kirkland, WA.

Municipal effluents are known to contain estrogenic substances that can induce vitellogenesis in both male and female fish species. In one of our recent studies, we found that bivalves exposed to a municipal effluent and its plume had induced alkali-labile phosphates (vitellins) and increased lipids in the gonad indicating effects by estrogens. Females were also found to be more responsive than males in this situation. We tested the hypothesis that mussels exposed to xeno-estrogens discharged from a municipal effluent over a complete cycle of reproduction (1 year) could alter their sex ratio in favor of females. *Elliptio complanata* mussels were exposed with two different cage designs: one cage was designed to hold mussels in a benthic pen while the other consisted of individualized mussels in nets attached to a PVC frame. The cages were firmly attached to the bottom with spikes and placed upstream (2 km) and downstream of the municipal effluent plume (7 and 10 km) in June 2001. The cages were collected in June 2002 for sex ratio and growth determinations.

The results showed that the benthic pen was a superior design because most mussels survived the long-term exposure period because mussels in these cages were able to rise over the accumulated sediments. Mussels collected at both downstream sites had an increased condition factor (i.e., g wet weight/length^3) and the proportion of females were significantly ($p<0.05$) increased at 7 km downstream (62% females) and 12 km downstream (67%) with respect to the upstream site (42% females). Our results suggest that prolonged exposure of freshwater mussels to municipal effluent discharges skews the sex ratio in favor of females and may have adverse effects on bivalve reproduction performance in the long term.

Developing an Ecological Risk Assessment Based Cumulative Assessment Strategy Using Caged Bivalves to Characterize Exposure and Effects. M.H. Salazar[1], S.M. Salazar[1], F. Gagne[2] and C. Blaise[2]. [1]Applied Biomonitoring, Kirkland, WA; and [2]Environment Canada, Centre Saint-Laurent, Montreal, QC.

There is a need to develop a meaningful monitoring strategy for assessing cumulative effects in freshwater and marine environments. While various stressor- and effects-based approaches have been proposed, proper use of the ecological risk assessment paradigm could provide all the requirements of cumulative effects assessment with equal emphasis on characterizing exposure and effects. Most previous environmental effects monitoring in Canada has focused on natural populations of fish, but caged bivalves can be used in the ecological risk assessment based cumulative assessment strategy as they offer a number of advantages with respect to experimental control, source identification, and establishing links between chemical exposure and biological effects.

A case study demonstrating the utility of this approach in the St. Lawrence River will be presented. It includes collaborative work conducted over the past four years between Applied Biomonitoring and Environment Canada scientists at the St. Lawrence Centre. A variety of monitoring and assessment strategies were combined to assess the City of Montreal's municipal effluent. Bioaccumulation of chemicals in mussel tissues has been effectively used to characterize internal exposure or dose, and mussel growth rates to characterize potential effects. Various bivalve biomarkers, endocrine disruption, reproductive effects, and feminization have been used to establish links between exposure and effects. Most exposure and effects endpoints measured in fish can be measured in bivalves.

Lions Gate Wastewater Treatment Plant Near-Field Habitat and Benthic Community Assessment Using Video Imaging Methods. A. Lewis[1], D. Hodgins[2], H.C. Bailey[3], M.D. Payne[4] and D. Swanston[5]. [1]Greater Vancouver Regional District, Burnaby, BC; [2]Seaconsult Marine Research Ltd., Salt Spring Island, BC; [3]EVS Environment Consultants, North Vancouver, BC; [4]Paine, Ledge and Associates, North Vancouver, BC; and [5]Seacology, North Vancouver, BC.

In its liquid waste management plan, the Greater Vancouver Regional District committed to environmental monitoring to aid in making management decisions concerning wastewater treatment and operational issues. As part of this process, a monitoring program was designed specifically for the Lions Gate Wastewater Treatment Plant (WWTP). One component of this program is a near-field, hard-bottom benthic community assessment in First Narrows to assess spatial effects of the effluent. A seabed video imaging technique (SIMS towed camera) was chosen because conventional infauna community structure analysis is not possible in the armoured substrate. Field surveying was completed in November 2001, providing continuous east-west coverage of the non-depositional regime in First Narrows. The video data were classified for the presence of vegetation (13 taxa), epifauna (60 taxa) and substrate, and yielded 1,252 independent data records, fairly evenly distributed over First Narrows. These records were then amalgamated into 170 observational units reflecting variations in distance, orientation, depth and substrate in the benthic communities, for statistical analysis. Three effluent exposure metrics were derived for each observational unit:

annual mean concentration, annual geometric mean concentration and annual average daily maximum concentration. Spearman rank correlations between biological variables and effluent concentrations were used as the basic measure of potential exposure effects. Non-metric multidimensional scaling was also used to examine relationships among six major faunal taxa. These statistical tests suggest that floral and faunal communities may be affected by the effluent discharged from the Lions Gate WWTP. Possible effects include a reduction in abundance and diversity of red- and green-bladed algae in the near-surface layer, and an increase in abundance and richness in anemone and sponge populations in the deeper portions of the channel.

Bioassay Variability and the Issue of Compliance. D. Vieira[1], M.E. Warren[1] and J. Harkness[2]. [1]City of Kamloops, Kamloops, BC; and [2]Urban Systems Ltd., Kamloops, BC.

Variability in the results of the LC50 96 hour rainbow trout test on the City's effluent resulted in extensive research to understand the cause. Data indicated that a seasonal variability existed in the Ph and ammonia concentration. The differences in the ammonia concentration was expected as the current treatment facility is not designed to nitrify. The variation in these two factors alone did not explain the variability in the test outcome. Several samples, taken at the same time, were sent to different accredited laboratories.
A large deviation in the results was observed, including 100% survival and 100% death. Based on the criteria that 6 deaths out of the 10 test fish is indicative of a test failure, in 8 tests completed at 4 different laboratories, the effluent failed in 3 of the tests and passed 5 of the tests. Further data analysis indicated that one test variability was the increase in pH throughout the test period, potentially resulting in conditions of ammonia toxicity. These fluctuations during the winter months, when nitrification is limited, are more likely to result in significant fish deaths and hence test failure. The variability in the outcome of the test is of critical importance to a municipality which must comply with the terms of the permit or the Municipal Sewage Regulation in relation to a non-toxic effluent.

Evaluation of Methods to Control pH Drift in the Rainbow Trout Acute Lethality- Test as it Applies to Municipal Waste Water Effluents. G.R. Schroeder and G.C. van Aggelen. Environment Canada, Pacific Environmental Science Centre, North Vancouver, BC.

Compliance testing of municipal waste water effluents (MWWE) has recently drawn considerable attention from federal regulators, municipal waste water departments, and the media due to the apparent inconsistency of Rainbow trout (RBT) bioassay results from different toxicology laboratories. The ability of the standard Environment Canada RBT acute lethality test (RM 9 and 13) to provide accurate results has been questioned as a result of the persistent test artifact of pH increase that occurs under standard aeration test conditions. Small increases in pH (i.e. 1.0 pH unit) causes a substantial increase in the quantity of toxic un-ionized ammonia (NH_3) present in a MWWE sample. The Pacific Environmental Science Centre has conducted research into treatment methods using zeolite, non-aeration and CO_2 gas to control the pH drift or eliminate un-ionized ammonia toxicity during RBT bioassay testing.

Bioassay results from several municipal waste water plants indicate that the application of a mixed blend of CO_2 gas to MWWE samples is an effective method to control pH drift while maintaining the integrity of the RBT test. The results from the bioassay testing conducted with different types of MWWE samples (lagoon, primary and secondary treatment) under standard aeration, non-aeration and CO_2 treatments will be presented.

A Science Based Approach to Determine the Need for Sewage Treatment: The Decision Process.

L.A. Taylor[1], T.C. Michelsen[2], P.L. Striplin[3] and C. Larose[1]. [1]Capital Regional District, Victoria, BC; [2]Avocet Consulting, Kenmore, WA; and [3]Caenum Environmental Associates, Settle, WA.

Throughout the 1990s monitoring results were used to argue that sewage discharged from the urbanized area of the Capital Regional District (CRD) did not require sewage treatment. However, there was no formal process where these data could be used to determine the need for sewage treatment. In 1998, the CRD and the then BC Ministry of the Environment, Lands and Parks established a workgroup to develop such a process for the seafloor environment off the CRD's two main sewage outfalls.

This work group produced the Trigger Process – an overall process that determines when the development of treatment facilities or source control measures are necessary to protect the receiving environment. This process is site specific and is based on three environmental receptors; the health of the benthic community, the health of the epibenthic community and bioaccumulation of contaminants into the food chain. The measurement endpoint for these receptors are total taxa, polychaete abundance, SDI, deep sea mussel tissue weight, shell length and chemicals levels in tissue. For each of these endpoints warning and adverse effects levels were developed. The Trigger Process is a decision framework that involves annual monitoring, comparison of monitoring data to warning levels, investigations into the sources/chemicals responsible for any exceedance, trend analysis to determine if adverse effects levels are likely to be exceeded in the next 7 to 10 years and the evaluation of source control and treatment alternatives to determine which alternatives best meet the goal of preventing adverse effects.

A Science Based Approach to Determine the Need for Sewage Treatment: The Monitoring Component.

C. Larose and L.A. Taylor. Capital Regional District, Victoria, BC.

A marine assessment program has been conducted at the Clover and Macaulay Point wastewater outfalls in Victoria, British Columbia, since 1988 to assess the effects of wastewater discharges on the receiving environment. The 2000 program included a seafloor monitoring component that consisted of chemical and benthic community samples at Macaulay Point and mussel tissue residue and population analyses at Clover Point. These studies were conducted to support a science based decision process termed the Trigger Process. Specific endpoints measured as part of this process included total taxa richness, polychaete abundance and SDI for benthic communities, tissue weight, shell length and chemical levels in tissue for the deep sea mussel populations. The 2000 data were used to test the Trigger Process and determine the health of the receiving environment. Data were compared to derived warning and effects levels. A weight-of-evidence approach was applied to determine if individual sampling stations exceeded these levels and to assess the spatial extent of the effects. Sediment chemistry data were also used in the overall weight-of-evidence analysis. Environmental effects were observed only within 200 m of the outfalls. The test trial of the Trigger Process met with some challenges. However, the main conclusions of this analysis were that site-specific biological endpoints are good indicators of wastewater discharge effects on the seafloor.

Use of Toxicity in Controlling Municipal Wastewater Effluents.

J.D. Clarke[1] and B. Munson[2]. [1]Environment Canada, Dartmouth, NS; and [2]Environment Canada, Edmonton, AB.

Four substances (ammonia, inorganic chloramines, nonylphenol and its ethoxylates, and textile mill effluents) were recommended for inclusion on the CEPA (*Canadian Environmental Protection Act*) List of Toxic Substances (Schedule 1) on June 23, 2001. This action initiated the risk management process for these substances. Since a major source for these substances is municipal wastewater effluents, the risk management process required consideration of CEPA obligations to publish a preventive or control instrument within 24 months, and suggested consideration of *Fisheries Act* obligations to prevent discharges of deleterious substances.

Environment Canada is proposing several major steps towards a comprehensive strategy for municipal wastewater effluents, that involves pollution prevention planning as a first step, to be followed by a common longterm national strategy and objectives developed jointly with other governments. As one example of this approach, the paper focusses on risk management for ammonia in municipal wastewater effluents, where the science must inform the policy and technical proposals that must be made. A risk management objective is proposed, and the arguments for various approaches to achieving the objective are discussed.

The proposed approach involves the derivation of a site-specific discharge limit that compares the total ammonia concentration in the effluent with the projected ammonia concentration in the receiving water, accounting for any pH change and no dilution. The lower concentration of the two that will be non-acutely lethal then becomes the site-specific discharge limit for ammonia, and will determine any need for ammonia control. This approach is included in the federal public consultation process on a comprehensive strategy for municipal wastewater effluents that is currently under way.

Site-Specific Water Quality Objectives for the *Canadian Enviromental Protection Act* - Municipal Waste Water Effluents Toxics Ammonia and Chloramines. P.-Y. Caux and S.S. Dixit. Environment Canada, Environmental Quality Branch, Ottawa, ON.

Municipal wastewater effluents are resulting in closure of shellfish beds, reduced tourism, health impacts, and beach closures in many places across Canada. Under its' Municipal Wastewater Strategy, Environment Canada has endorsed the use of site-specific water quality objectives for protecting aquatic environments from the discharge of toxic contaminants. We have derived water quality objectives (WQO) for ammonia and chloramines for wastewater effluents. In addition to the current Canadian Water Quality Guideline (CWQG) for ammonia, four different WQO derivation procedures were evaluated: [1] Background Concentration Procedure, [2] Recalculation Procedure, [3] Water Effect Ratio Procedure, and [4] Resident Species Procedure.

In the case of ammonia, the Recalculation and Resident Species Procedures provide the best opportunities for establishing site-specific WQOs. Derivation of site-specific WQOs for ammonia is recommended in situations where ammonia concentrations in receiving waters are close to, or slightly above, the WQGs. For chloramines, the Recalculation procedure was recommended. The CWQG matrix of numbers (pH vs DOC) could also be used where site-specific data are lacking. When assessing the impacts of wastewater effluent, these site-specific objectives are most effective when couched within a proposed Environmental Quality Objectives Framework which will be included in the presentation.

Environmental Quality Objectives Framework for Municipal Wastewater Effluent. K. Hedley[1], B.W. Kilgour[2], G.M. Pastershank[1], J. Arnott[1] and D.J. Spry[1]. [1]Environment Canada, Environmental

Quality Branch, Ottawa, ON; and [2]Jacques Whitford Environmental Ltd., Ottawa, ON.

All levels of government in Canada have the responsibility for the management of municipal wastewater, as well as preventing or minimizing the harmful impacts of municipal wastewater effluents on the environment. Four substances that are primarily found in municipal wastewater effluents (ammonia, inorganic chloramines, textile mill effluents, and nonylphenol and its ethoxylates) are proposed to be added to Schedule 1, List of Toxic Substances under the *Canadian Environmental Protection Act (1999).*

Environment Canada is proposing to use pollution prevention planning as the first step towards developing a comprehensive strategy to deal with the CEPA-toxic substances and the broader issues connected with municipal wastewater. Within this strategy, Environment Canada is committed to using environmental quality as one of the drivers for risk management actions. Environmental quality objectives are intended to help determine the ability of risk management actions to adequately protect the Canadian environment. As a result, a proposed Environmental Quality Objectives Framework for municipal wastewater effluents has been developed. Toxicity, chemical, and biological objectives are the three key components linked in this framework. This presentation will discuss the proposed framework and how it fits into Environment Canada's comprehensive strategy on municipal wastewater effluents.

Aeration with CO_2-Supplemented Air as a Method to Control pH drift in Toxicity Tests. J.R. Elphick[1], M.C. Hindle[1], H.C. Bailey[1] and S. Bertold[2]. [1]EVS Environment Consultants, North Vancouver, BC; and [2]Greater Vancouver Regional District, Burnaby, BC.

The Greater Vancouver Regional District routinely conducts required acute toxicity tests using Environment Canada rainbow trout protocols on discharges from the District's wastewater treatment plants (WWTPs). Samples from the Annacis and Lulu WWTPs have exhibited toxicity in these tests. The within test toxicity has been confirmed to be ammonia, but is caused by an increase in test pH that is associated with the aeration of test solutions. Ammonia toxicity is caused primarily by the unionized form (NH_3) and increases significantly with increasing pH. Therefore, an increase in sample pH caused by aeration of wastewater samples under test conditions can have a profound effect on the toxicity of ammonia. Because the elevated pH observed in these tests is generally uncharacteristic of the waste stream, it can be considered an artifact of the toxicity test design.

This study was performed to evaluate a method for stabilizing the pH of wastewater effluent samples, which are at circum-neutral pH, by testing with trout and aerating with air supplemented with CO_2. The results indicated that this procedure can be effectively applied to acute toxicity tests performed with rainbow trout to prevent upward drift of sample pH during exposure. It also has application to Toxicity Identification Evaluations in terms of maintaining sample pH at desired levels.

Toxicity Identification Evaluation Case Study of a Publicly-Owned Wastewater Treatment Plant Located in Imperial Valley, California, US. J. Rudolph, C. Stransky and A. Farmer. AMEC Earth and Environmental, San Diego, CA.

A municipal sewage treatment plant located in the Imperial Valley has historically exhibited toxicity in their quarterly NPDES monitoring using the fathead minnow *Pimephales promelas* and the Cladoceran *Ceriodaphnia dubia.* Previous Phase I Toxicity Identification Evaluation (TIEs) performed in 1999 and 2001 identified ammonia as a primary toxicant of concern. Variations in the sensitivity of the two test species over time, however, indicate that more than one toxicant may be responsible for toxicity depending on the sampling period. In addition, concern that ammonia may be masking other toxicants of concern prompted a complete Phase I through Phase III TIE investigation in the spring of 2002. TIE procedures once again identified ammonia as a primary toxicant of concern. Surfactants and the trace metal copper were identified as secondary constituents of concern although confirmation procedures indicated that they were not contributing to any of the observed toxicity in the single spring 2002 sample. Plant upgrades are currently in the planning stages to address these constituents of concern and continued monitoring will evaluate their effectiveness.

Sewer Use Bylaws – a Review of the Science and Practical Relevance for Source Control. P.M. Campbell. ToxEcology – Environmental Consulting Ltd., Vancouver, BC.

Sewer Use Bylaws can be an effective means to control sources of specific contaminants entering the environment. When sewer use bylaw restrictive waste limits are based on relevant ecological and human risk assessments and practical risk management considerations they can be effective instruments to promote pollution prevention. To determine the effectiveness and relevance of current sewer use bylaws the scientific basis underlying the restrictive waste limits in the recently updated Greater Vancouver Regional District (GVRD) and BC Capital Regional District (CRD) Sewer Use Bylaws was reviewed. The aspects considered in this assessment included: [1] Worker safety, [2] WWTP process inhibition, removal efficiency and design capacity, [3] WWTP permit discharge levels, [4] Influent/Effluent flow, [5] Biosolids recycling issues, [6] Initial dilution at the outflow, [7] Water quality objectives, and acute and chronic toxicity etc.

A number of important data gaps were identified in the scientific rationales underlying some of the restrictive waste limits. For example, the scientific rationale behind the derivation of the restrictive waste limit for PAHs raised a number of important questions that should be addressed. In addition, for some contaminants the sources that can be controlled by the current sewer use bylaws contribute <10% of the total loadings to sewers – indicating that alternative risk reduction strategies need to be explored as a priority.

Ecological Survey of the South Saskatchewan River Downstream of the Saskatoon Wastewater Treatment Plant. M. Constable. Environment Canada, Environmental Protection Branch, Edmonton, AB.

In 1996, the City of Saskatoon rebuilt their wastewater treatment plant a state-of-the-art tertiary treatment system removing much of the biochemical oxygen demand (BOD), suspended solids, nutrients and minerals. In order to provide information to Environment Canada's risk management process for these substances regarding the possible benefits from a significant upgrade to a treatment system, Environment Canada's Edmonton office decided to conduct an ecological survey of the conditions downstream of the City of Saskatoon's wastewater discharge four years after its upgrade.

The results show that the benthic macroinvertebrates have responded in a positive manner, albeit in a subtle way. There were more sensitive species in the river than found previously. The insect community of the river did not conform to what is typically found below a sewage discharge high in organic matter, nor was there a pattern of community alteration between the upstream control site and the downstream potentially affected sites. The concentrations of chlorophyll-*a* from attached algae were fairly high 150 meter below the discharge; however, the density of the algal growth declined rapidly downstream. Growths of algae did not appear to be excessive anywhere. The heavy macrophyte growths of previous years were not present. Water chemistry downstream of the discharge was not significantly different from upstream. Overall, the City of Saskatoon does not appear to be having a significant ecological impact on the South Saskatchewan River.

The Biodegradability of Ionic and Neutral Polymers. D.J. Porter, S. Falicki, E. Karalis and G. Hammond. Environment Canada, New Substances Branch, Hull, QC.

Biodegradation is an important factor for determining whether or not a substance may persist in the environment and is used frequently when conducting ecological risk assessments of polymers. Polymers, unlike discrete chemicals, may contain several functional groups on branched or repeating chains. If such polymers degrade in the environment, smaller fragments of lower molecular weight could be produced which may behave differently or elicit ecological effects not seen in the main polymer. The degradation of polymers can depend on many factors such as intrinsic properties and the nature of the surrounding environment. It can also vary depending upon the medium in which it is released. For this work we have examined the degradability of ionic and neutral polymers using data received at the New Substances Branch of Environment Canada. Degradability with respect to polymer type, average number molecular weight (Mn), percentage of low molecular weight oligomers, and water solubility is discussed.

Aquatic Fungi Living in Polluted Groundwater Habitats. G. Krauss[1], K.R. Sridhar[2], K. Jung[3], J. Ehrman[4] and F. Bärlocher[4]. [1]Groundwater Microbiology Junior Research Group, UFZ Centre for Environmental Research, Leipzig/Halle, Germany; [2]Department of Biosciences, Mangalore University, Mangalore, India; [3]Department of Chemical Ecotoxicology, UFZ Centre for Environmental Research, Leipzig/Halle, Germany; and [4]Department of Biology, Mount Allison University, Sackville, NB.

The decomposition of allochthonous organic material in freshwater ecosystem is initiated by aquatic hyphomycetes (AQH). A special situation exists in metal-rich biotopes, resulting from man's activities. Such habitats are found in Central Germany. Weathering of slag heaps and dumps resulted in groundwater, lakes and streams with extremely high metal concentrations (Krauss et al. (2001) Nova Hedwigia 72: 419-428; Sridhar et al. (2001) Aquat. Micro. Ecol. 26: 73-80). Even heavily polluted surface waters had a surprisingly high fungal diversity, and alder leaves were actively colonized and degraded by AQH (Sridhar et al. (2001) Aquat. Micro. Ecol. 26: 73-80).

Here we extend our studies to underground habitats contaminated with heavy metals and organic pollutants. We exposed sterile leaves to water from groundwater wells, and checked them for colonization by aquatic hyphomycetes. Fungal species numbers and spore productions were compared with concentrations of pollutants and standard physico-chemical parameters of the emerging groundwater. A Pollen Tube Growth Test (PTG Test) representing a simple assay for estimation of the cytotoxicity was applied. The procedure used is suitable to assess the hazardous potential of aqueous environmental samples. Our study shows for the first time that an amazing diversity of AQH and other aquatic fungi occur in native and polluted groundwater habitats.

Developing a Benthic Cage for Long-Term, *In-situ* Tests with Freshwater and Marine Bivalves.

M.H. Salazar[1], S.M. Salazar[1], F. Gagne[2], C. Blaise[2] and S. Trottier[2]. [1]Applied Biomonitoring, Kirkland, WA; and [2]Environment Canada, Centre Saint-Laurent, Montreal, QC.

Abstract

Environment Canada's St. Lawrence Center and Applied Biomonitoring conducted four separate cooperative transplant studies with caged freshwater mussels (*Elliptio complanata*) to assess exposure and effects from a Montreal Urban Center (MUC) effluent. Bivalve biomarkers, including vitellin, have been developed to establish links between chemical exposure (characterized by tissue chemistry) and associated biological effects (characterized by mussel growth rates). Vitellin is the bivalve counterpart to vitellogenin in fish. In order to verify the hypothesis that prolonged exposure to estrogens in municipal effluent could induce feminization, caged mussels were deployed upstream and downstream of the MUC effluent diffuser for one year, which encompassed a complete mussel reproductive cycle. Two different cage designs were tested. The first cage, an epibenthic cage, utilized standard mesh bags with compartmentalized cells. The epibenthic cages were placed directly on top of the sediments and held with cinder block anchors. The second, a benthic cage, was buried in sediment with only the top exposed. The benthic cage consisted of a plastic tub with an internal mesh chamber. Mussels were confined by this mesh insert and there were no individual compartments. Sediment was retained by the solid outer walls. Mussel survival was much higher in the benthic cages (83%) than in the standard cages (9%). The benthic cage trapped sediment and the mussels moved to these surficial sediments. This preliminary sex-reversal test has shown that feminization in freshwater mussels can be experimentally induced. A significantly higher number of females was found downstream than upstream. We believe this is the first time that such a phenomenon has been induced in caged bivalves. Evolution of this cage design will be discussed with respect to long-term sediment exposures in depositional environments. The rationale for development of this cage and possible applications will also be presented.

Why Develop a Benthic Cage for Bivalves?

One might ask the question, "Why develop a benthic cage for bivalve testing?" A benthic cage can be used to provide more realistic characterizations of exposure and effects for several different applications, including: [1] Validating laboratory studies. Characterizing exposure may be the most critical element in ecological risk assessment because an inappropriate interpretation of exposure can diminish the significance of characterizing effects. Recognition of this concern has caused a shift from laboratory toxicity tests to mesocosms and field studies where environmentally realistic exposures are easier to achieve. It is also helpful to characterize effects under environmentally realistic conditions in the field. These are both critical elements to ecological risk assessment; [2] Assessment of long-term exposures and associated effects. Currently, the most subtle effects can only be manifested after long-term exposures under environmentally realistic conditions. Field observations by Environment Canada scientists had shown a higher percentage of females downstream of a City of Montreal municipal effluent than upstream. Environment Canada wanted to determine if sex reversal could be experimentally induced in the field under environmentally realistic conditions. For this reason, it was necessary to include the mussel's entire reproductive cycle to induce these effects and thus a long-term exposure period of one year; [3] Characterizing benthic exposure pathways. Several studies conducted by Applied Biomonitoring have shown a statistically significant relationship between chemicals found in bivalves suspended above the bottom or on bottom sediment, chemicals in sediment, and various effects endpoints. Nevertheless, other scientists have questioned whether or not the pathways of exposure, bioaccumulation, and associated biological effects would be the same in bivalves just above or on bottom sediment as in those living in bottom sediment; and [4] Supplementing laboratory bioaccumulation tests. Questions have also been raised with respect to the ability of the standard 28 day *Macoma* bioaccumulation test conducted under laboratory conditions adequately represents "real-world" conditions in marine benthic communities. These questions are primarily attributable to the relatively short-term exposure, the ability of *Macoma* and other bivalves to remain closed for extended periods of time, and potentially unrealistic laboratory exposures. Using the benthic cage in marine environments would help validate the results of laboratory bioaccumulation tests. A comparable 28 day laboratory test has been developed for freshwater using the standard freshwater bivalve test organism *Corbicula fluminea.*

Cage Development

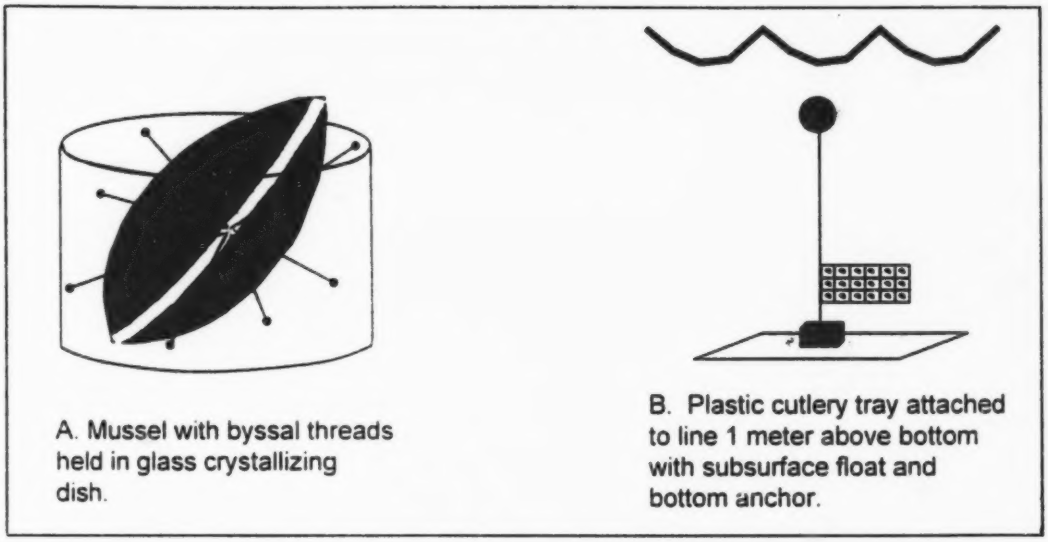
From aquaria to mesocosms to the field: it all started in the lab as a toxicity test. Between 1971 and 1973 we developed and applied a laboratory bioassay using byssal thread production as an effects endpoint. This work was based on initial studies conducted by Don Reish. Between 1973 and 1977 we developed a prototype cage using glass crystallizing dishes in a plexiglas frame to facilitate counting byssal threads as we had done in the lab (Fig. 1A). This was the conceptual beginning of a bivalve cage with individual compartments to facilitate multiple effects measurements on the same individuals. The next step in the development process, between 1985 and 1986, was the use of plastic ice cube trays drilled to maximize water circulation for the mussels while they were held in mesocosm tanks. After demonstrating that the caged juvenile mussels deployed at the seawater intake for the mesocosm grew almost four times faster than those in the mesocosm tanks, we focused on field transplant studies. We began by using plastic cutlery trays for these field transplant studies (Fig. 1B). These cages held more test animals than ice cube trays and provided more room for growth. Measurement endpoints were bioaccumulation and growth.

From rigid cages with individual compartments to flexible cages with individual compartments. In 1991 we conducted a mussel transplant in Puget Sound at the Harbor Island Superfund Site. It was the first combination of a rigid cage (plastic cutlery tray) with individual compartments and a flexible mesh cage with 10 mussels per compartment. Juveniles in the compartmentalized cages were used primarily for growth measurements and the adults in the flexible mesh bags for bioaccumulation.

A similar approach was used in San Diego Bay in 1993, although in this case extra mussels were used for evaluating various bivalve biomarkers. It quickly became apparent that the flexible mesh cages offered the most versatility in terms of holding a wider size range of test animals and maintaining individual compartments. In addition, the mesh is inexpensive, disposable, and does not have to be cleaned. The first flexible mesh cage with a PVC frame (Fig. 2A) was used for monitoring tissue chemistry and growth in Sinclair Inlet, in the vicinity of the Puget Sound Naval Shipyard. All of these studies could be considered water column studies, even though the Harbor Island mussels and Sinclair Inlet mussels were deployed 1 meter above the bottom as shown in Fig. 1B. We were able to demonstrate a statistically significant relationship between chemicals in mussel tissues, chemicals in sediment, and mussel growth rates.

Freshwater Bivalves on Sediment.

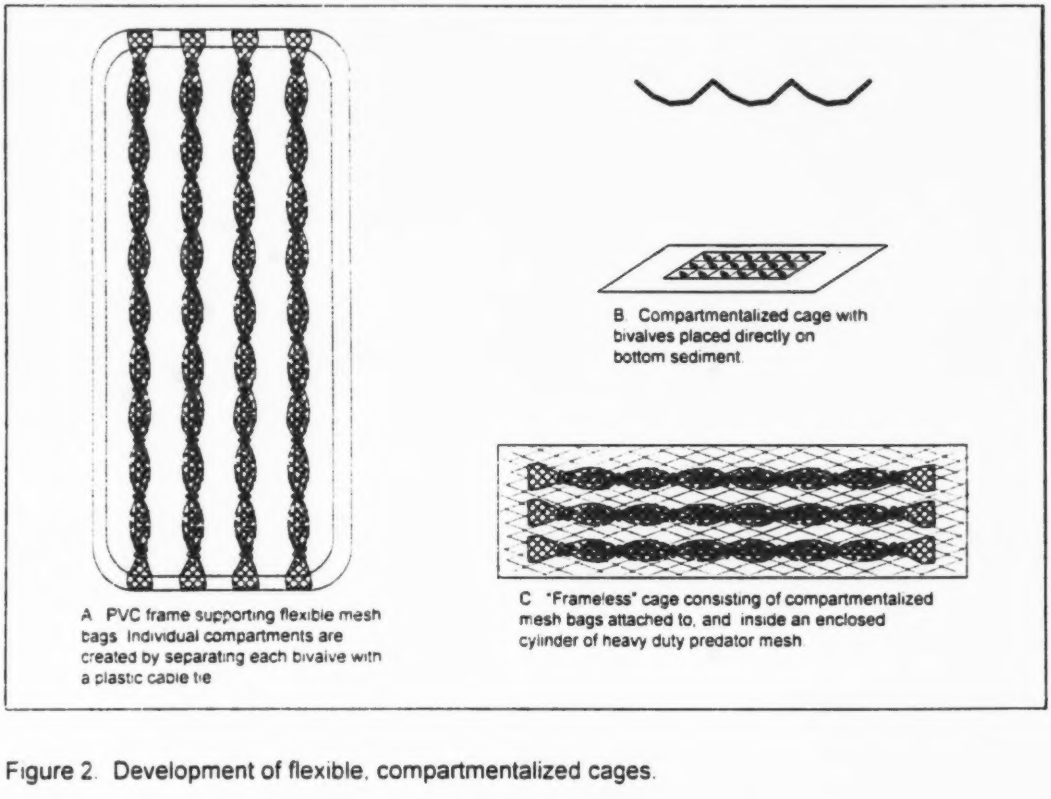
In 1996 we conducted our first freshwater caged bivalve study at the Nyanza Superfund site on the Sudbury River, MA to evaluate the bioavailability of mercury. Freshwater mussels (*Elliptio complanata*) caged in the flexible mesh bags in a PVC frame (Fig. 2B) were transplanted directly on bottom sediment. Results showed that methylmercury (MeHg) was biologically available all along the reach of the Sudbury River being tested. Although measured MeHg concentrations decreased down stream from the Superfund Site, growth rate measurements showed that when the concentrations were normalized to account for growth dilution there was no statistically significant



A. Mussel with byssal threads held in glass crystallizing dish.

B. Plastic cutlery tray attached to line 1 meter above bottom with subsurface float and bottom anchor.

Figure 1. Early development of compartmentalized mussel cages.



A. PVC frame supporting flexible mesh bags. Individual compartments are created by separating each bivalve with a plastic cable tie.

B. Compartmentalized cage with bivalves placed directly on bottom sediment.

C. "Frameless" cage consisting of compartmentalized mesh bags attached to, and inside an enclosed cylinder of heavy duty predator mesh.

Figure 2. Development of flexible, compartmentalized cages.

difference with distance from the contaminated site. This study helped confirm the importance of measuring growth to help interpret tissue chemistry results.

Caged clam (*Corbicula fluminea*) studies in were conducted in 1997 and 2000 at the Cannelton. Superfund site in Sault Ste. Marie, MI to evaluate the effectiveness of remedial activities. Clams were transplanted to 10 sites in Tannery Bay, an area used for disposal of tannery waste products, and two reference sites on the St. Mary's River. Again, caged clams were placed directly on the bottom as shown in Fig. 2B. Tissue chemistry results showed that Cr bioavailability had decreased over time in most areas. A statistically significant relationship was found between Cr in sediment and Cr in mussel tissues. MeHg was not biologically available.

Applied Biomonitoring was contracted by GR Craig & Associates to help design and conduct freshwater monitoring studies in 2001 and 2002 to characterize polychlorinated biphenyl (PCB) exposure in the Speed River, Guelph, Ontario. The study required caging bivalves in small channels with less than 1 foot of water during the summer. This requirement was just as challenging as deploying caged mussels at a depth of 200 meters in Port Valdez. The "frameless" cage design (Fig. 2C) was used to place mussels within the concave structure of these small streams. The logistics of this study were very complicated and required continued coordination with the Ministry of Environment (MOE) and local freshwater bivalve experts. *Lasmigona costata* and *Elliptio complanata* were used as test species. Other obstacles included high summer temperatures, low oxygen at a treatment site, and the presence of natural predators and other mammals that disturbed the sediment in the study area.

Background for the Urban Effluent Study

General. Bivalves have been shown to be an appropriate test organism with internal systems similar to fish. Previous studies have shown that mussels downstream of the MUC had higher concentrations of vitellin than upstream mussels and long-term exposures of fish to estrogenic chemicals has led to increased number of females. Environment Canada scientists at the St. Lawrence Center chose to develop bivalve biomarkers and conduct sex reversal tests because bivalves have many internal systems, such as an endocrine systems, which are similar to fish and because bivalves are easier to collect, cage, and measure. Applied Biomonitoring previously developed an American Society for Testing and Materials (ASTM) Standard Guide for bivalve cages and has extensive experience and expertise in using bioaccumulation and growth to characterize exposure and effects in marine, estuarine, and freshwater bivalves. Caged bivalves have also been accepted by Environment Canada as an alternative to the adult fish survey for Environmental Effects Monitoring (EEM) at pulp and paper mills in Canada. Other scientists have used benthic cages and it was felt that development of such a cage would be relatively simple.

There was also some concern that mussels would not survive one year in the traditional mesh cage on the bottom and that the mussels would need to be buried in sediment rather than on top of sediment to survive. Environment Canada scientists had expressed concern that mussels in the traditional mesh cages deployed on bottom sediment in the St. Lawrence River would be subjected to extremely low temperatures outside of their tolerance limits. Results would later suggest that sediment burial and not temperature caused high mortality in the mesh cages but nevertheless, it was still a concern. Subsequent observations on the Grand River in Guelph, Ontario would demonstrate that mussels on cobble bottoms that could not bury in sediment could survive low winter temperatures for many years.

Biomarkers. Environment Canada scientists at the St. Lawrence Centre have been studying the

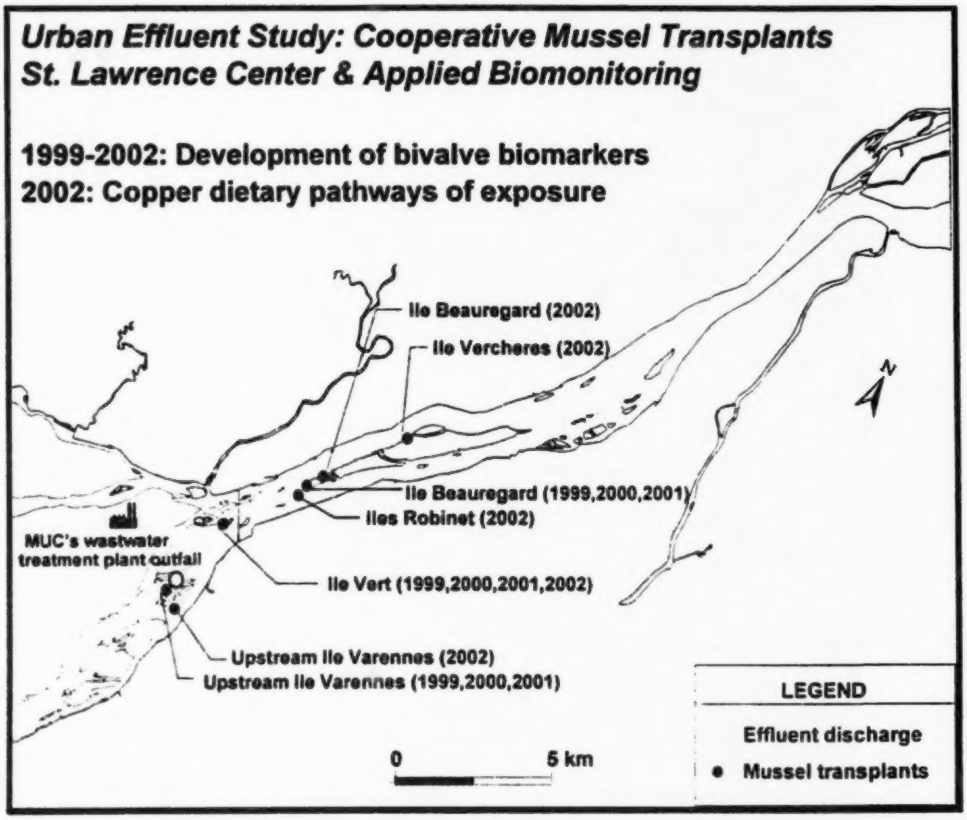

Urban Effluent Study: Cooperative Mussel Transplants
St. Lawrence Center & Applied Biomonitoring
1999-2002: Development of bivalve biomarkers
2002: Copper dietary pathways of exposure
Ile Beauregard (2002)
Ile Vercheres (2002)
Ile Beauregard (1999,2000,2001)
Iles Robinet (2002)
MUC's wastwater treatment plant outfall
Ile Vert (1999,2000,2001,2002)
Upstream Ile Varennes (2002)
Upstream Ile Varennes (1999,2000,2001)
N
0
5 km
LEGEND
Effluent discharge
● Mussel transplants

Figure 3. Map showing locations of the effluent discharge and mussel transplant stations in the Montreal effluent study.

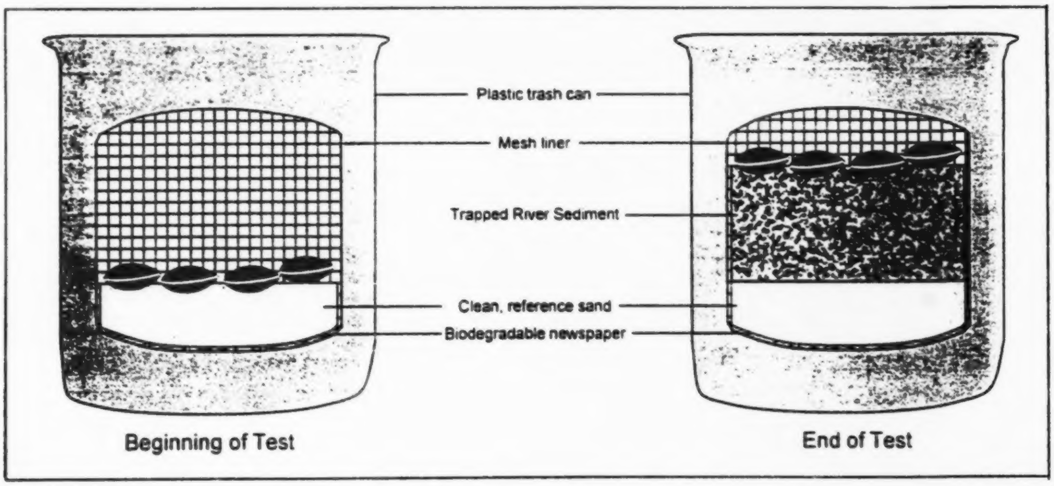

Plastic trash can
Mesh liner
Trapped River Sediment
Clean, reference sand
Biodegradable newspaper
Beginning of Test
End of Test

Figure 4. Diagram of the benthic cage at the beginning and at the end of the 1-year exposure period.

effluent from the MUC wastewater treatment plant outfall for several years and during the past four years have conducted cooperative mussel transplant studies with Applied Biomonitoring. The purpose of these studies was to combine the traditional approach of measuring bioaccumulation and growth of transplanted bivalves with bivalve biomarkers. The wastewater treatment plant, the effluent dispersion plume, and mussel transplant sites are shown by year in Fig. 3. As part of this monitoring program Environment Canada scientists observed higher concentrations of female *Elliptio complanata* downstream of the effluent than upstream and wanted to determine if sex reversal could be experimentally induced in controlled field experiments to complement the bivalve biomarker monitoring and measurements of increased vitellin production in downstream mussels. This was the major impetus for the development of this benthic cage.

Dietary Exposure Pathways. In a complementary study, the St. Lawrence Center and Applied Biomonitoring are working together to characterize exposure and effects associated with the effluent plume and metals, particularly Cu. A major element of that study includes characterizing exposure from water and dietary pathways of exposure. As shown in Fig. 3, this work only began in 2002. As in the organic portion of the study, bivalve biomarkers are being combined with measurements of bioaccumulation and growth. However, in this pathway exposure study, uptake of metals is being measured in several different organs including gill, digestive gland, gonad, and remaining tissues. Metals in the gill will be used as a surrogate for water exposure pathways and in the digestive gland for dietary exposure pathways. Although not developed specifically for this purpose, the benthic cage could also be used for this complementary exposure pathway study for metals. Benthic exposure pathways need more study to help characterize and understand processes, evaluate the importance of exposure from water, sediment, and food, and to determine if water and sediment quality criteria based on laboratory exposures are adequately protective of various species under "real world" conditions.

Methods for Urban Effluent Study

Cage design. The overall design of the benthic cage (Fig. 4) includes an inner mesh chamber, held in place with plastic cable ties, that holds the clean sand and test mussels at the beginning of the test. The benthic cage utilizes biodegradable newspaper that covers the bottom of the inner mesh chamber to help retain the clean sand. All mussels were measured for length and weight at the beginning of the test and surrogate mussels were sexed. Mussels were evenly distributed on top of the clean sand in the benthic chamber before deployment

Deployment. Two types of mussel cages were deployed to compare their suitability for long-term exposures in highly depositional environments on the St. Lawrence River. The traditional mesh cages were held upright in the water column with floats and secured to the river bottom with cinder block anchors as shown in Fig. 1B. The benthic cage was buried up to about 5 cm of the top and secured to the river bottom with stainless steel stakes. Divers helped support the cage as it was lowered to the bottom by a winch and then buried and secured the cage.

Retrieval. Pingers were attached to cages prior to deployment. Acoustic receivers fixed on the pingers were used to locate the cages. A boat winch and divers were again used to raise the benthic cage out of the water. Once on board, the top mesh was removed and the mussels were removed from the sediment. The number of surviving mussels was recorded during the removal process. After removal they were held in ice chests until they were returned to the laboratory and held in flow-through laboratory tanks. During removal, it was observed that the mussels had migrated to the surface of the trapped sediment (fine, dark mud) after initially being placed on top of the fine, clean sand (Fig. 4). This was obvious because of the difference in color and texture of

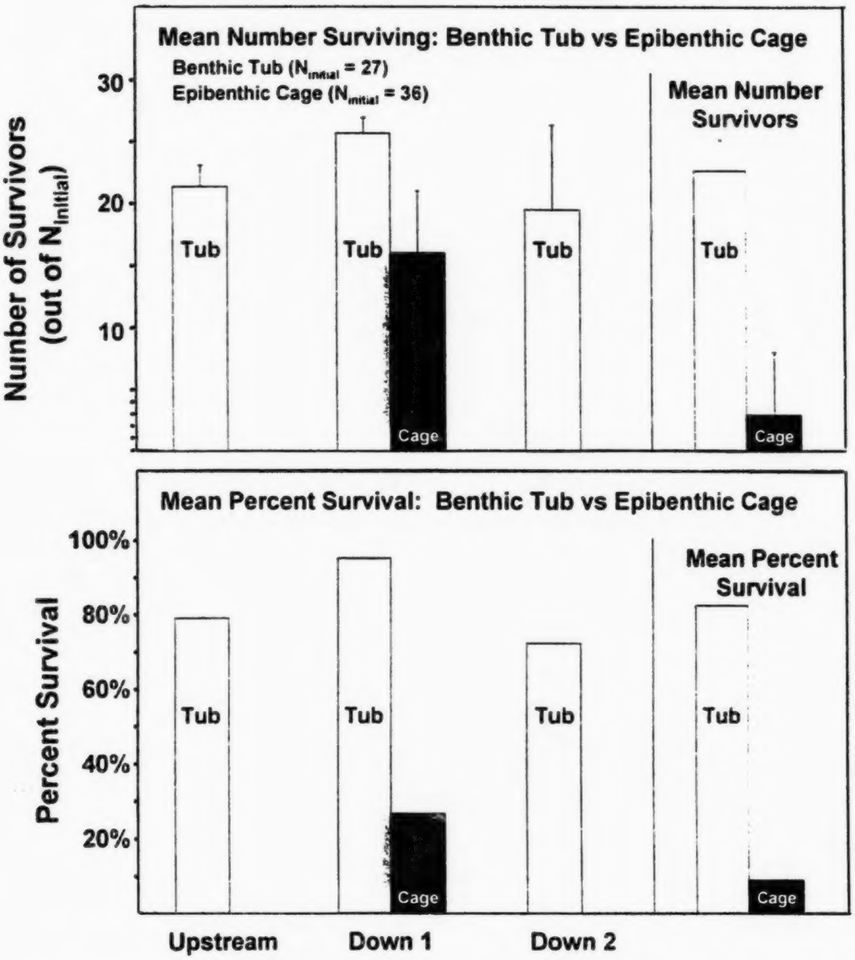


Figure 5. Mean number of mussels surviving and mean percent survival at the end of the test.

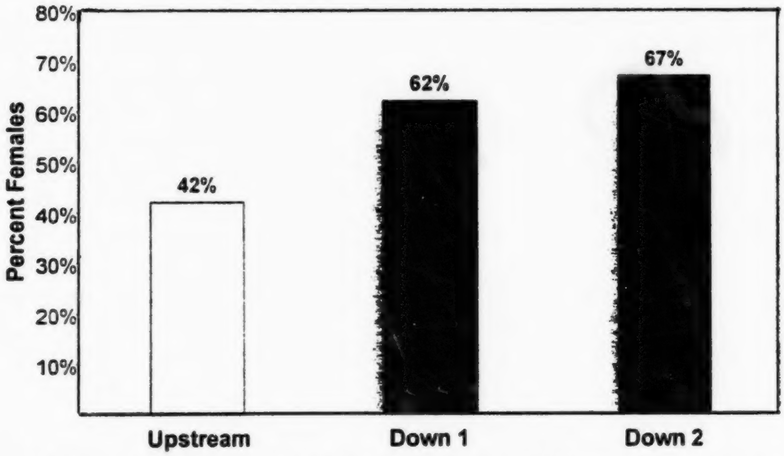


Figure 6. Percent females at upstream and downstream stations after 1-year exposure.

the sand and the mud. Figure 4 also shows the plastic mesh retainer on the inside of the benthic cage or "corral."

Laboratory measurements. After overnight holding in flow-through laboratory tanks, the number of survivors in each cage was confirmed and several growth metrics measured including whole animal wet weight, shell length, tissue weight, and shell weight. Digital calipers and portable analytical balance connected to a PC were used to record the measurements into an Excel spreadsheet. Tissues were removed for weighing, chemical analysis, and biochemical analysis. Gonads were separated for sex determination and biomarker analysis.

Results: Freshwater bivalves in sediment – the Benthic Cage
Mussel survival was significantly higher in the benthic cages (83%) than in the standard cages (9%) as shown in Fig. 5. The number of females was significantly higher in downstream mussels (62% and 67%) than in upstream mussels (42%) as shown in Figure 6. The benthic cage trapped suspended sediment, and the mussels migrated up into these surficial sediments (Figure 4). This preliminary sex-reversal test has shown that feminization in freshwater mussels can be experimentally induced in a controlled field experiment with a long-term, 1 year exposure period. The benthic cage was successful in achieving high mussel survival, directly exposing the mussels to both contaminated water and contaminated sediment, and demonstrating that sex changes could occur in the field downstream of a municipal effluent and have effects on mussel reproduction.

General. Survival was significantly higher in the buried benthic tubs than in the epibenthic mesh cages. We believe this is primarily attributable to burial of the epibenthic cages by sediment. Sediment was trapped in the benthic tubs and exposed the caged mussels to contaminated water and sediment.

Survival. Survival by benthic tub ranged from 59% to 100% and by station from 59% to 93%; average survival was 83%. Survival by epibenthic mesh cage ranged from 0 to 44% and by station from 0 to 26%; average survival was 9%. Survival of freshwater mussels (*Elliptio complanata*) was significantly higher in the benthic tubs than in the epibenthic cages. Feminization. There was a higher percentage of females at the downstream stations (62% and 67%) than at the upstream station (42%). The cages provided a method for characterizing long-term water and sediment exposures and experimental induction of sex changes. These results confirmed preliminary observations by Environment Canada of a higher percentage of females than males in the natural populations downstream from the MUC.

Summary: The test was successful. Survival after a 1-year exposure period was high. Increased feminization was observed. All cages were retrieved in good condition. Contaminated sediment was trapped in the cage. Mussels migrated to the contaminated sediment.

Conclusions: The benthic cage was a success. More robust than mesh cages for long-term tests. Useful for many different bivalve species. Supplement to laboratory bioaccumulation tests. Can be used to study exposure pathways. Particularly useful in highly depositional areas.

Applications: The benthic cage is a potentially powerful tool. Caging bivalves facilitates characterizing exposure and effects and any clinical measurements. Controlled field experiments combine experimental control & environmental realism. Long-term exposures to evaluate subtle phenomena such as feminization are possible. Ability to experimentally induce phenomena observed in the field. Validation of laboratory testing.

Tissue Chemistry of Horse Mussels (*Modiolus*) Monitored off the Clover Point Wastewater Outfall, Victoria, British Columbia. S.A. Lyons[1] and M.D. Paine[2]. [1]Environmental Services, Capital Regional District, Victoria, BC; and [2]Paine, Ledge and Associates, North Vancouver, BC.

A spatially extensive population of horse mussels (*Modiolus)* resides adjacent to the Clover Point deep municipal wastewater outfall in Victoria, British Columbia. This population has been utilised as a monitoring tool since the mid-1990's to assess the potential effects of the outfall on the marine-receiving environment. Mussel tissue chemistry and population assessment studies have been conducted on a regular basis as part of the Capital Regional District's Marine Assessment program. Since 2000, mussels have been collected in the fall of every year from several monitoring stations and an associated reference station. Mussel tissues are analysed for metals, polycyclic aromatic hydrocarbons, phthalate esters, chlorinated hydrocarbons, base-neutral extractables and chlorinated phenolics.

Results from these analyses indicate that Cu and Pb are the only contaminates that occur at elevated concentrations in tissue near the discharge, and quickly decrease in concentration with distance from the outfall. Ag and Hg concentrations increase with distance from the outfall. This phenomenon called reverse gradient is partially attributable to growth dilution. Growth dilution will occur whenever the tissue weight increases faster than the uptake of contaminant. Organic contaminants are rarely detected at the detection limits utilised by the CRD, but nevertheless will continue to be monitored on a yearly basis.

Wastewater Treatment Polymers: A Real or Perceived Risk to Aquatic Organisms? S.J. de Rosemond[1], K. Liber[1] and A. Wilson[2]. [1]Toxicology Centre, University of Saskatchewan, Saskatoon, SK; and [2]Environment Canada, Environmental Protection Branch, Yellowknife, NT.

Water soluble polymers have been used within water and wastewater treatment facilities since the mid-1950s to clarify potable water, industrial effluents and municipal wastewater, thicken and de-water sludge, and aid in the filtration of primary and digested sludge. The primary function of these polymers in water clarification and sludge de-watering processes is to destabilize colloidal suspensions and induce particle flocculation. Residual wastewater treatment polymers in effluents were initially thought to be of little toxicological concern to aquatic organisms due to their high molecular weight ($>10^4$) and the propensity of these compounds to bind to suspended solids. However, recent research using weight-of-evidence approaches has demonstrated that wastewater treatment polymers were responsible for both the acute and chronic toxicity of effluents to aquatic organisms.

The present problem is that current Toxicity Identification Evaluation (TIE) procedures are unable to identify and confirm polymers as the toxic component in effluents. In addition, there are no routine analytical detection methods available that are selective and sensitive enough to detect polymers in wastewater effluents at concentrations that are toxic to aquatic organisms. Furthermore, very little information exists on individual polymer formulations with regard to toxicity to aquatic organisms, environmental persistence, and degradation pathways. Therefore, in order to determine if wastewater treatment polymers pose a real risk to aquatic organisms, data quantifying the degree of toxicity to exposed organisms must be generated, the environmental fate of polymers and their degradation products must be determined, and adequate analytical detection methods need to be developed.

Basis of Metal Uptake and Toxicity

Session Chair: K. Liber

Silver Uptake in Rainbow Trout (*Oncorhynchus mykiss*) and Gulf Toadfish (*Opsanus beta*) at Different Salinities. J.W. Nichols[1] and R.C. Playle[2]. [1]Department of Biology, University of Waterloo, Waterloo, ON; and [2]Department of Biology, Wilfrid Laurier University, Waterloo, ON.

To replace water lost by osmosis, marine and euryhaline fish drink seawater, making the gut a target for metal interactions. We generated data on Ag binding to gills and gut of rainbow trout first acclimated in the laboratory to various salinities (0.05% to 100% seawater) and then exposed to 0.5 μM $AgNO_3$, with or without organic matter (10 mg C/L total organic carbon (TOC), added as Aldrich humic acid). The protective effects of organic matter (OM), normally important in freshwater, were eliminated at higher salinities.

Ag tended to accumulate in the posterior intestine, and carbonate minerals (mixed $CaCO_3$ and $MgCO_3$ pellets), thought to have an osmoregulatory function, formed in intestines of trout acclimated to higher salinities (>40% seawater). A similar experimental protocol was used for marine gulf toadfish: 1 μM $AgNO_3$ exposures, with or without OM (10 mg C/L TOC, added as natural Suwannee River OM), in 2.5% to 100% seawater. Overall, toadfish results were similar to those from trout, where Ag accumulation and the effects of OM were reduced in brackish water to seawater conditions due to increasing chloride concentrations (e.g., Ag^+ complexation by Cl^-).

Quantitative Measurements of Metal Transfer from Stable Isotopically Labelled $^{67}Zn_7$metallothionein IIb to Apo-Metallonzyme Substrates. A.Z. Mason[1], R Moeller[2], K. Thrippleton[1] and N. Perico[1]. [1]Department of Biological Sciences, California State University, Long Beach, CA; and [2]Department of Physiology and Biophysics, University of California, Irvine, CA.

The ability of metallothionein (MT) to act as a donor of metals for the activation and inactivation of various apo-metalloenzymes has been studied using coupled HPLC-ICP-MS. The specific donation of Zn from MT was quantified by monitoring the transfer of ^{67}Zn to recipient apo-enzymes from mono-isotopically labelled $^{67}Zn_7$MTIIb. Transfer was independently confirmed by assaying for a concomitant increase in enzymatic activity. Catalytic reactivation was quantitatively consistent with the appearance of ^{67}Zn on both apo-alkaline phosphatase and apo-carbonic anhydrase and the stable isotopic signatures demonstrated negligible transfer of extraneous Zn from sources of contamination. In contrast, incubation of apo-carbonic anhydrase with CdMTIIb caused only minor transfer of Cd which, quantitatively, accounted for only 1.5% of the original content of metal (Zn and Cu) associated with the holo-enzyme. Thus, although these two metals have comparable chemistries (electronegativity, ionic radius and coordination characteristics) and can substitute for each other in the â-domain of MT to form Cd_5Zn_2MTII with mixed metal clusters, apo-CA can clearly differentiate between the two metals and shows a higher degree of selectivity for Zn over Cd.

It is concluded that although MT under certain cellular conditions can release a number of non-essential metals such as Cd, Hg and Ag, the specificity shown by apo-metalloenzymes, such as carbonic anhydrase, for their native metal prevents the sequestration of these alternate metals into catalytic centers and thereby limits their potential for toxic interaction through competitive substitution.

Ongoing Molecular Characterization of a Metal-Binding, Histidine-Rich Glycoprotein (HRG) in Marine Mussel, *Mytilus edulis*, Blood Plasma. W.E. Robinson[1], M. Sugumaran[2], G. Wallace[1], A. Abebe[2], M. Gaudette[1] and S. Catanzano[2]. Departments of [1]Environmental, Coastal and Ocean Sciences, and [2]Biology, University of Massachusetts Boston, Boston, MA.

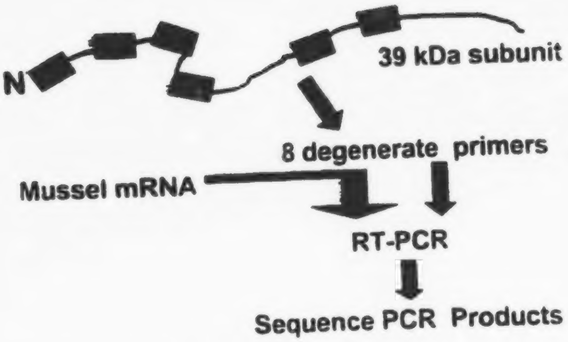
Work in my laboratory is aimed at elucidating metal transport mechanisms in the blood of various marine invertebrates, particularly bivalve molluscs. Working with ^{109}Cd as well as stable Cd, we have identified several blood plasma proteins in *Mytilus edulis* that bind Cd and other metals, although apparently nonspecifically (Robinson et al., 1997). Cd binds particularly strongly, however, to one set of three bands (35, 37 and 39 kDa) on 7.5% SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE). Cd is retained even after electrophoresis. We now consider these three bands to be polymorphic forms of the same protein subunit.

We have purified this Cd-binding protein using ammonium sulfate precipitation, Imobilized Metal Ion Affinity Chromatography (IMAC) and DEAE cellulose chromatography (Nair and Robinson, 1999). When the purified protein was spiked with ^{109}Cd and analyzed by native PAGE, a single protein band was observed, and virtually all the radioactivity was associated with this band. Using Fergusson Plots, we have determined that this single protein has a MW of 63 kDa. When the purified protein was spiked with ^{109}Cd and analyzed by SDS-PAGE, radioactivity was restricted to the 35, 37 and 39 kDa bands. A broad diffuse band at 29 kD did not bind Cd. This 29 kDa band is either a degradation product of the other bands, or a discrete subunit of the intact, 63 kDa native protein. Additional SDS-PAGE gels were stained with Schiff's reagent to detect carbohydrate. The 35, 37 and 39 kDa bands stained positive, whereas the 29 kDa band did not stain (Nair and Robinson, 1999).

This Cd-binding protein has been tentatively identified as a Histidine-rich Glycoprotein (HRG; Nair and Robinson, 1999, 2001a) and has a number of characteristics that are similar to mammalian HRG (Table 1). It's molecular weight, isoelectric point and carbohydrate content are similar to human HRG. It has a very high histidine content (13.6%), as does human HRG, although its proline content (3.2%) is much lower and its methionine content (2.5%) much higher than the human protein.

Table 1. Similarities and differences between Histidine-rich Glycoproteins (HRG) from *Mytilus edulis* and humans.

Measured Attribute	HRG_{mussel}	HRG_{human}
Molecular weight	63 kDa	66 kDa
Isoelectric point	4.2-5.8	5.6-6.2
Carbohydrate	12%	14%
Cd log *K* (assuming 1 class of binding sites)	4.2	4.9
Histidine	14%	13%
Plasma distribution	~60%	<1%
Proline	3.2%	12.9%
Methionine	2.5%	0.4%



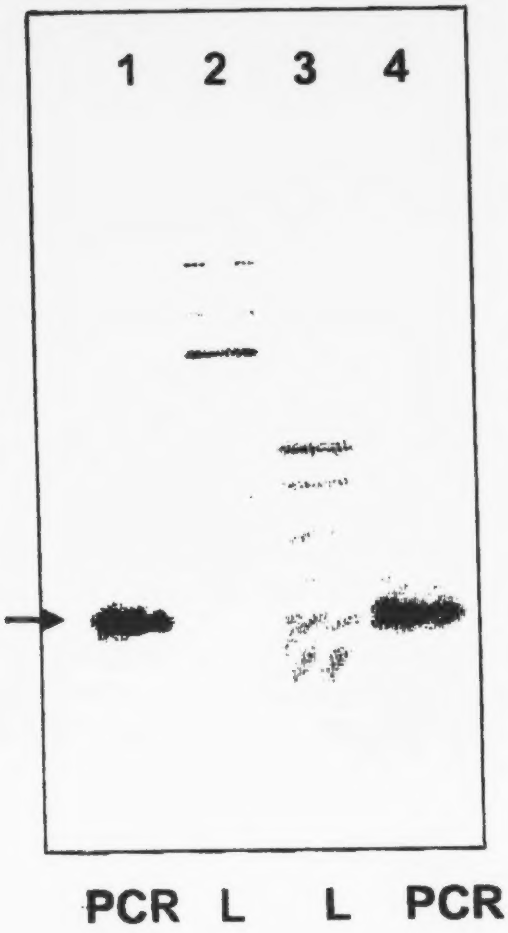


Fig. 1. Experimental design for determining cDNA sequence of *M. edulis* Hisidine-rich Glycoprotein using Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR), starting with small amino acid sequences obtained by Edman degradation of trypsin digests of the 39kDa subunit of a protein.

Fig. 2. Example of a Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) experiment using degenerative primers to *Mytilus edulis* Histidine-rich Glycoprotein (HRG). Arrow points to a 250 bp PCR product (lanes 1 and 4). Lanes 2 and 3 contain two separate bp size and concentration markers.

It's log *K* for Cd binding, assuming a single class of binding sites, is similar to that reported for human HRG under the same assumption. However, we have shown that the binding of Cd to mussel HRG is best characterized by a 2-class binding site model, with the stronger affinity class having 6 binding sites per molecule and a log *K* of 7.65, and the lower affinity class having 10 sites per molecule and a log *K* of 5.41 (Nair and Robinson, 2001a). Similar to mammalian HRG, mussel HRG binds a variety of nonessential and essential metals (Ca, Cd, Ni, Hg, Pd, Zn). Unlike mammalian HRG which constitutes <1% of the blood plasma proteins, mussel HRG comprises ~60% of the proteins present in mussel blood. Thus, mussel HRG, as an analogous if not a homologous

Table 2. Preliminary partial cDNA sequence (327 bp) of *Mytilus edulis* Histidine-rich Glycoprotein (HRG), obtained from Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) using degererative primers and mRNA obtained from pooled gill, mantle and gonad tissue. This prelimiary sequence codes for the N-terminal of the 39 kDA subunit of mussel HRG.

..TAATACGG	CGGTTGATNA	TCACCATGAT	GACCACCACG	ATGCTCCGAT
AGTTGGCCAC	CATGACGCTT	TCCTTGAGGC	CGAATTCGAT	TTATCATTCC
TCGATGCTGT	CCTTGAATAC	TTCATCCACC	ACGTCATCGA	AAAAGAGATT
CTCGATGTTG	AAAACCATAC	TGAGCATAAT	AACCATGAGA	TTGTCGCCCT
CCATCTACAA	ATTAATCATC	TGCTCGAGGA	GGTCGAATAT	TTCATATCTC
ACCACGTGGC	ATTTTCTGCT	GNNCTGAACT	CTCCTNTTGN	TNACATNNTC
GCTGATGAAA	TCANCCCNTT	CTNNAANAA.		

protein, may be a good model system for trying to understand the role of HRG in humans. We have already shown that it functions as a metal transport protein in mussels, mediating the rapid transfer of Cd from mussel blood to the kidney (Nair and Robinson, 2001b).

In order to better understand mussel HRG's function, we have begun molecular work to determine its cDNA sequence (Fig. 1). Automated Edman degradation was conducted on both the N-terminal of the 39 kDa SDS-PAGE band and on five additional segments of this subunit obtained by trypsin digestion in order to determine partial amino acid sequences. From these six sequences, we were able to design eight degenerative primers (six forward primers and two reverse primers; Fig. 1). Using purified mRNA extracted from a mixture of mantle, gonadal and gill tissue (Qiagen mRNA Tissue Isolation kit) as a template for Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR; Qiagen RT-PCR kit), and using four combinations of forward and reverse primers, we have, thus far, obtained three overlapping PCR products (100 bp, 250 bp and 300 bp segments out of an estimated total of 1170 bp). Each RT-PCR reaction product was checked for purity on 2% agarose gels (e.g., Fig. 2 depicts a single 250 bp PCR product). Our best results were obtained with degenerative primers that had a difference in Tm of <2.5°C. We have not been successful in utilizing oligo dT as a reverse primer in our RT-PCR preps.

After cleanup of our 100, 250 and 350 bp reaction products (Qiagen PCR Cleanup kit), samples were sequenced on an ABI Prism 377 DNA sequencer, using the same degenerative primers that were originally used in the RT-PCR reactions. Our preliminary results, using both forward and reverse sequencing, allowed us to piece together a 327 bp cDNA sequence (Table 2) that codes for the N-terminal of the mussel HRG. We estimate that this is 28% of the of the total cDNA of mussel HRG. We were able to successfully translate this cDNA sequence back to an amino acid sequence (~90 amino acids) that had a high percentage of histidine (13%), with a number of paired histidine sequences. More importantly, we could readily identify the N-terminal amino acid sequence that we had obtained by Edman degradation.

Once we have obtained the full cDNA sequence of mussel HRG, we will be conducting experiments to determine where the protein is produced in the mussel, whether it is ubiquitous throughout the bivalve molluscs, whether it can be induced or regulated, and whether it or portions of its gene sequence are homologous with mammalian genes. Funded by National Sea Grant 5710001173.

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Modelling Toxicity of Nickel to *Hyalella azteca* using a Biotic Ligand Model Approach. J.E. Schroeder[1], U. Borgmann[2] and D.G. Dixon[1]. [1]Department of Biology, University of Waterloo, Waterloo, ON; and [2]Environment Canada, National Water Research Institute, Burlington, ON.

Short term (7 day) bioaccumulation experiments using adult *Hyalella azteca* were performed to determine Ni uptake in media of different characteristics. Ni concentrations in tissue were related to concentrations of competing ions (Ni, Ca, Mg, Na, K and H) in solution using a Michaelis-Menton form of equation. Transformation of this equation allowed estimation of apparent binding constants by linear regression, with X as the concentration of an ion in solution and Y as the ratio of the concentration of Ni in water to that in tissue. To reduce the influence of outliers and data variability, constants were also calculated using the median method, which estimates each constant as the median value of all possible combinations of X and Y. Bioaccumulation was related to toxicity by comparing tissue concentrations predicted by the model under given exposure conditions with those corresponding to observed LC50s in exposures of young *Hyalella* (less than 10 days old). The relatively good fit of the model showed that biotic ligand modeling is possible in *Hyalella* and the median method is a suitable method for estimating binding constants.

Arsenic Toxicity, (As(III) vs. As(V), to Three Benthic Invertebrates Under High and Low Dissolved Oxygen Conditions. E.C. Irving[1], K. Liber[1], J.M. Culp[2], R.B. Lowell[2], Q. Xie[3], R. Kerrich[3] and C. Casey[2]. [1]Toxicology Centre, University of Saskatchewan, Saskatoon, SK; [2]Environment Canada, National Water Research Institute, Saskatoon, SK; and [3]Department of Geological Sciences, University of Saskatchewan, Saskatoon, SK.

In aquatic environments, arsenic (As) speciation is governed by abiotic factors (e.g., redox potential, dissolved oxygen) and biotic factors (e.g., bacteria). Exposure primarily occurs in the form of two inorganic species, As(III), As(V)), which may differ in toxicity. During toxicity testing, As speciation is generally assumed to remain stable throughout the exposure period, even though As(III) has been shown to oxidize to As(V), and As(V) reduce to As(III). In addition to speciation, low dissolved oxygen (DO) condition may also represent a confounding factor in toxicity testing. This study assessed the significance of speciation on As toxicity alone, and in combination with low DO stress to the benthic invertebrates, *Hyalella azteca*, *Chironomus tentans* and *Baetis tricaudatus*. In addition to As toxicity, speciation and bioaccumulation, bacteria numbers and physicochemical variables were determined in 10 to 12 day static-renewal tests. As(III) exerted greater toxicity on all benthic invertebrates compared with As(V). Although not always conclusive, the results indicated that exposure to As(III) under hypoxic stress may exacerbate As(III) sublethal toxicity. In some tests, As(III) oxidized to As(V) between water changes, likely caused by bacteria. Thus, biotic oxidation of As(III) may also be an important confounding factor in As toxicity testing.

Endocrine Disrupting Compounds

Session Co-chairs: M.M. Vijayan and P.M. Campbell

Reproductive and Histopathological Effects of Developmental Estrogenic Exposure in Zebrafish. D.M. Janz[1], L.P. Weber[2] and R.L. Hill, Jr[2]. [1]Department of Veterinary Biomedical Sciences and Toxicology Centre, University of Saskatchewan, Saskatoon, SK; and [2]Department of Zoology, Oklahoma State University, Stillwater, OK.

We determined concentration dependent effects of 4-nonylphenol (NP) and 17α-ethinylestradiol (EE) on sex ratios, vitellogenin induction, gametogenesis, breeding success, and organ histopathology in zebrafish (*Danio rerio*). Fish were exposed from 2 to 60 days post-hatch (dph) to NP (10, 30, or 100 μg/L nominal), EE (1, 10, or 100 ng/L nominal), or solvent control. The percentage of phenotypic males at 60 dph changed from 45% (9/20) in solvent controls to 0% at 10 ng/L EE and 10% at 100 μg/L NP.

Exposure to NP (≥100 μg/L nominal) and EE (≥1 ng/L nominal) caused concentration dependent suppression of gametogenesis in both male and female zebrafish. Severe kidney pathology was observed in 60 dph zebrafish, specifically glomerular dilation or degeneration, fibrosis, tubule enlargement and tubule necrosis, at a threshold of 10 ng/L EE. However, minor kidney histopathology indicated by increased pyknotic nuclei in kidney tubule and interstitial (hematopoietic) cells was detected at lower estrogenic exposures (≥10 μg/L NP nominal) than delayed gametogenesis. The sex ratios of adults determined after grow-out in clean water from 60 to 160 dph revealed no significant departure from 1 male:1 female, suggesting that estrogenic exposure during sexual differentiation did not irreversibly alter phenotypic sex. Despite this, breeding trials conducted in adult fish revealed significant reductions in the percent of viable eggs, hatchability, and swim-up success at 10 ng/L EE and 100 μg/L NP. We conclude that functional rather than morphological changes may be more important for future evaluations of developmental exposure to xenoestrogens in fish.

Effects of Ethynylestradiol on Early Development of Amphibians in a Boreal Lake. B. Park[1], K.A. Kidd[2] and J.G. Eales[1]. [1]Department of Zoology, University of Manitoba, Winnipeg, MB; and [2]Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, MB.

Amphibians are exposed to endocrine-disrupting chemicals that may ultimately contribute to population declines. The current study addresses the effects of a known hormone mimic on aspects of anuran development. A study lake was dosed with 17α-ethynylestradiol (EE2) at a mean concentration of 6 ng/L, in June to September 2001 (Experimental Lakes Area, NW Ontario, Canada). Tissue thyroid hormone concentrations were assessed in cage-reared green frog tadpoles (*Rana clamitans*) from the EE2-treated lake and two reference lakes. Mean T3 and T4 concentrations were not significantly different among lakes. Growth and development rates of cage-reared green frog tadpoles were not significantly different among lakes, whereas hatch success was lower (though nonsignificant) on the EE2-treated lake. Gonads of wild-caught mink frog tadpoles (*R. septentrionalis*) and cage-reared green frog tadpoles were examined histologically. Low frequency of intersex was detected in tadpoles from the EE2-treated lake, no intersex occurred in tadpoles from the reference lakes. Few impacts of environmentally relevant concentrations of EE2 were observed for these anuran species.

The ING Tumor Suppressor Protein is Regulated by Thyroid Hormone in the *Xenopus laevis* Tadpole and is Affected by the Herbicide Acetochlor. M.J. Wagner and C.C. Helbing. Department of Biochemistry and Microbiology, University of Victoria, Victoria, BC.

The tumor suppressor protein, ING, has previously been shown to play a role in regulating programmed cell death (apoptosis), cell cycle progression, chemosensitivity and senescence. Very little is known about the role and mechanism of action of ING, especially during development. In our lab, ING was discovered in frogs. To study ING in the context of normal development, amphibian metamorphosis is used as a model system. Diverse processes occur simultaneously, including apoptosis, differentiation, and proliferation. Thyroid hormone (TH) is solely responsible for triggering tadpole metamorphosis into a frog. Precocious induction of metamorphosis results in an elevation of ING protein and transcript levels in tissues with different metamorphic fates. Moreover, inhibition of TH-induced apoptosis of the tail abrogates the TH-induced accumulation of ING protein. Therefore, ING appears to be important in the hormonal response and may determine cellular outcome. Currently, we are investigating whether a known endocrine disrupting compound, the herbicide Acetochlor, has an effect on ING expression. Acetochlor is known to accelerate TH-induced metamorphosis and alter gene expression. Preliminary data show that ING expression is affected by Acetochlor.

Effects of Gemfibrozil, a Pharmaceutical in the Canadian Environment, on Nulcear Peroxisome Proliferator-Activated Receptor Expression Levels in Goldfish. C. Mimeault, V. Trudeau and T.W. Moon. Center for Advanced Research in Environmental Genomics (CAREG), Department of Biology, University of Ottawa, Ottawa, ON.

Pharmaceuticals are reported in the aquatic environment at concentrations that can exceed µg/L levels in sewage treatment plant effluent waters. To date, most of the literature available is limited to the environmental occurrence of pharmaceuticals rather than to their fate or effects on non-target organisms. Among these are chemicals known as peroxisome proliferators (PPs) that have been shown to increase the size and number of peroxisomes, increase the rate of β-oxidation and induce the formation of liver tumors in rodents. The effects of PPs are mediated through the nuclear peroxisome proliferator-activated receptor (PPAR).

Two doses (10 and 100 mg/kg) of gemfibrozil (GEM), a lipid and cholesterol lowering fibrate drug that acts as a PP, was injected every second day for 8 days into goldfish (*Carassius auratus*). This acute exposure to GEM allowed demonstrating its bioactivity in a non-target species. A significant 2-fold increase in serum glucose level ($p<0.05$) and a 54% triglyceride reduction ($p<0.05$) were observed. Furthermore, preliminary results suggest over 60% increase in mRNA PPAR-alpha isoform with the high dose treatment. These results would suggest that goldfish is also sensitive to PPs and that chronic environmental exposure might have biological effects.

A Potential Endocrine Disruptive Effect of Toxaphene on the Pituitary of Yellowtail Flounder (*Limanda ferruginea* Storer). G.E. Fåhræus-Van Ree and A. Brophy. Department of Biology, Memorial University of Newfoundland, St. John's, NF.

The histological and immunocytochemical effects of dietary Toxaphene were investigated on pituitaries of laboratory-bred sexually immature female and male yellowtail flounder from the same year-class (0^+, *Limanda ferruginea* Storer). The fish were fed twice daily for two weeks with uncontaminated food or food contaminated with hexane (the solvent for Toxaphene), or with one of three concentrations of Toxaphene (0.002, 0.02 and 0.2 mg/kg fish/day). Following the experimental period, the females were heavier than males, while males were generally more sexually mature than females. Computerized image analysis of the immunochemical reactions, as measured by area of fluorescence, revealed that the hormone level in the amphiphilic corticotrophs located in the rostral pars distalis was affected by Toxaphene, while that in the acidophilic somatotrophs and basophilic gonadotrophs located in the proximal pars distalis was not.

Fish exposed to the highest concentration of Toxaphene had significantly more adrenocorticotropic hormone (ACTH) /pituitary area (0.154±0.058) than the blank control group (0.018±0.005; $p=0.043$), indicating a possible dose-related stress response to Toxaphene. The males in four of the five studied groups had higher ACTH levels than the females, indicating a potentially higher sensitivity of males to Toxaphene. The increased synthesis and/or inhibited release of ACTH may result in changing plasma cortisol levels, which in turn may affect many metabolic body functions.

The Northern Rivers Ecosystem Initiative Endocrine Disruptors Research Program. M.E. McMaster[1], L.M. Hewitt[1], C. Portt[2], N. Denslow[3], G.R. Tetreault[1] and G.J. Van Der Kraak[4]. [1]Environment Canada, National Water Research Institute, Burlington ON; [2]C. Portt and Associates, Guelph, ON; [3]University of Florida, Gainesville, FL; and [4]Department of Zoology, University of Guelph, Guelph, ON.

Over the last 3 years we have been examining wild fish within the Northern Rivers Basin in Alberta for evidence of reproductive endocrine disruption. In the systems examined, fish are exposed to municipal sewage wastes and pulp and paper mill effluents. In our first year of study, wild fish downstream of the discharge of a municipal sewage treatment plant demonstrated some evidence of altered reproductive fitness.

Longnose sucker exposed to sewage effluent had altered circulating steroid hormone and vitellogenin levels as well as increased levels of hepatic oxidative stress, but showed no changes in gonadal development or levels of gonadal apoptosis. This discharge however, is fairly close (10 km) to the input of effluent from a bleached kraft mill and separating the effects was difficult, although mixed function oxygenase enzymes were induced to a greater extent in fish downstream of the pulp and paper mill. Studies in our third year focussed on trying to confirm the results that we had demonstrated earlier, as well as trying to separate out the effects of the two discharges. These studies compare reproductive endpoints in wild fish to endocrine activity accumulated in semi-permeable membrane devices from the two effluent sources relative to an upstream reference site.

Physiological and Biochemical Responses of Small Fish Exposed to Athabasca Oil Sands Sediment. G.R. Tetreault[1,2], M.E. McMaster[2], D.G. Dixon[1] and J.L. Parrott[2]. [1]Department of Biology, University of Waterloo, Waterloo, ON; and [2]Environment Canada, National Water Research Institute, Burlington, ON.

Fish collected from the Alberta Athabasca Oil Sands watershed, including sites off the oil sands deposit itself, were examined. Within two tributaries, fish are either unexposed (reference), exposed to naturally occurring oil sands related compounds. Wild fish collections and a laboratory sediment exposure were initiated to evaluate the influence of naturally occurring oil sands related compounds on reproductive function (gonadosomatic indices, fecundity, *in vitro* steroid production capacity) and hepatic responses (7-ethoxyresorufin-*O*-deethylase [EROD] activity).

In vitro gonadal incubations demonstrated lower levels of steroid production at the tributary sites within the oil sands deposit. Hepatic 7-ethoxyresorufin-*O*-deethylase (EROD) activity, as an indicator of exposure to oil sands related compounds, was elevated 5-fold at these same sites. In the laboratory, slimy sculpin (*Cottus cognatus*) were exposed to sediment samples from the Steepbank River sites at concentrations of 10 or 20 g/L (wet weight), for 4 and 8 days. For the purpose of evaluating EROD induction in fish exposed to oil sands sediment, a time period of 4 days was sufficient to induce what appeared to be maximum induction in this species by these sediments. The EROD activity measured in exposed fish was comparable to that measured in fish native to the oil sands area. This study as designed was not capable of predicting an altered ability of gonadal tissue of exposed fish to produce steroid hormones *in vitro*, as was demonstrated in the wild fish assessment of 1999 and 2000. Future development may further compromise the reproductive health of fish residing in this area.

Assessment the Estrogenic Potency of Effluents from Petrochemical Facilities and a Petroleum Refinery in Ontario. J.P. Sherry[1], T. Trepanier[1], C. Tinson[1], T. Moran[2], T. Kierstead[2] and S. Munro[3]. [1]Environment Canada, National Water Research Institute, Burlington, ON; [2]Sarnia Lambton Environmental Association, Sarnia, ON; and [3]Pollutech EnviroQuatics, Point Edward, ON.

In a previous study we demonstrated that wastewater from an Ontario Refinery could induce vitellogenin (Vg), a biomarker of exposure to estrogenic chemicals, in juvenile rainbow trout. In the present study we sought to reassess the estrogenic potency of the wastewater from that refinery and to also assess the estrogenicity of wastewater from three petrochemical facilities. The effluents were tested in a 21 day static renewal test in which the effluent was fully replaced daily. A competitive binding ELISA was used to detect induced Vg. A replicated tank design was used to allow statistical testing for tank effects. St. Clair River water from upstream of the industrial facilities was used as a negative reference. Waterborne 17ß-estradiol served as the positive control treatment. The results show that the wastewater from the petroleum refinery induced Vg in the treated fish. The wastewater from the petrochemical effluents did not induce detectable levels of Vg in the treated trout. We shall consider these data with respect to the industrial process at each facility and, in the case of the refinery, the potential for possible responses in feral fish.

Effects of Endocrine Disrupters on Seawater Adaptability, Growth and Survival of Atlantic Salmon Smolts. S.B. Brown[1], K. Haya[2], L.E. Burridge[2], J.G. Eales[3], D.L. MacLatchy[4], J.T. Arsenault[4], T. Jardine[4], R.E. Evans[5], B.K. Burnison[1], J.P. Sherry[1], T.D. Bennie[1] and W.L. Fairchild[6]. [1]Environment Canada, National Water Research Institute, Burlington, ON; [2]Department of Fisheries and Oceans, Biological Station, St. Andrews, NB; [3]Department of Zoology, University of Manitoba, Winnipeg, MB; [4]Department of Biology, University of New Brunswick, Saint John, NB; [5]Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, MB; and [6]Department of Fisheries and

Oceans, Gulf Fisheries Centre, Moncton, NB.

We previously identified relationships between historical applications of an insecticide containing 4-nonylphenol (4-NP) and catch data for Atlantic salmon (*Salmo salar*) populations. To test the hypothesis that 4-NP impairs parr-smolt transformation, we exposed Atlantic salmon smolts to pulse doses of water-borne 4-NP (4 to 100 μg/L). As positive control for estrogenic responses, we also exposed smolts to estradiol (E2) (100 ng/L). We monitored smolt response to a seawater challenge and subsequent growth in seawater. We assessed osmoregulatory competence, indicators of energetic balance, reproductive steroids, the estrogenic marker vitellogenin, and hormonal regulators of growth (e.g., insulin-like growth factor, thyroid hormones, thyroid hormone deiodinases, and thyroid histology).

There were no treatment related increases in mortality during a seawater challenge soon after exposure, however, growth in seawater was impaired in 25 to 35% of fish from various treatment groups (5% in control). Field studies suggest that exposure of smolts to Miramichi River water in 2000 and 2001 could also impair subsequent seawater growth. If these effects are due to steroidogenic potential, then steroidogenic activity stemming from various effluent sources may influence present day salmon populations.

Waste Products of Oilsands Mine Inhibit Sex Steroids in Exposed Fish. A. Lister[1], V. Nero[2], A.J. Farwell[2], G.J. Van Der Kraak[1] and D.G. Dixon[2]. [1]Department of Zoology, University of Guelph, Guelph, ON; and [2]Department of Biology, University of Waterloo, Waterloo, ON.

Oilsands mining by Syncrude Canada Limited (SCL) in northern Alberta generates the waste products: mature fine tailings (MFT) and tailings pond water (TPW). MFT is a toxic aqueous suspension of particles, organic acids, bitumen, and metals and TPW is a saline solution containing organic and inorganic contaminants. The purpose of this study was to examine the impact of MFT and TPW on reproductive steroid production in sexually mature goldfish, *Carassius auratus*. The goldfish (12 to 15 per pond per sex) were caged for 19 days in experimental ponds (0.16 ha each) constructed on SCL land and lined with or without MFT and capped with or without TPW. Three ponds were used in the study: pond 1 (no MFT, no TPW), pond 3 (MFT, no TPW), and pond 5 (MFT, TPW).

Plasma levels of testosterone (T) and 17β-estradiol (E_2) in male and female fish caged in ponds 3 and 5 were significantly ($p<0.05$) reduced compared with fish in pond 1 (control). Respectively, the T and E_2 (ng/ml) levels for males were: pond 1 (17.6±1.9, 0.38±0.03), pond 3 (2.2±0.4, 0.22±0.02), and pond 5 (2.7±0.3, 0.21±0.008). Respectively, the T and E_2 (ng/ml) levels for females were: pond 1 (10.6±0.4, 0.71±0.1), pond 3 (1.9±0.2, 0.28±0.02), and pond 5 (2.1±0.2, 0.29±0.03). *In vitro* testis and ovarian incubations were conducted on fish from the test ponds to evaluate potential differences in basal steroid production levels and responsiveness to the gonadotropin, hCG (10 IU/ml). Gonadal tissues of fish from all ponds responded similarly to hCG indicating that the oilsands wastes did not affect the capacity of gonads to produce steroids (T) under hormonal stimulation. Basal levels of T were reduced significantly in males and females from pond 5 compared with pond 1 indicating that the steroid inhibition may be caused at a site within the gonad. Collectively, these studies demonstrate that waste products of oilsands mining disrupt the reproductive endocrine system in goldfish.

Behavioural and Sex Ratio Modification of Japanese Medaka in Response to Environmentally Relevant Mixtures of Three Pesticides. K.L. Teather, C. Jardine and K.L. Gormley. Department of Biology, University of Prince Edward Island, Charlottetown, PE.

We exposed Japanese medaka (*Oryzias latipes*) to environmentally relevant concentrations of azinphos-methyl, chlorothalonil, endosulfan, and mixtures of all three to determine if combinations of these pesticides result in additive, less-than-additive, or more-than-additive effects. Medaka were exposed from fertilization to 7 days post-hatching and endpoints included survival, time to hatch, size at 7 days post-hatching, activity levels (as measured by distance swam) and foraging ability at 3 weeks post-hatching, and adult size, liver somatic index, and sex ratio at 5 months post-hatching.

While exposure to individual pesticides or pesticide mixtures did not affect survival, hatching time, or foraging ability, fry exposed to azinphos-methyl were significantly smaller at one week of age, and those exposed to chlorothalonil and the combination of chemicals showed reduced activity levels. Adult sex ratios were biased towards females in all groups exposed to pesticides, with those having been exposed to azinphos-methyl, chlorothalonil, and the pesticide mixture showing significant departures from an even sex ratio. There was no evidence of additive or more-than-additive effects of pesticide mixtures and we conclude that, for these chemicals, effects of exposure are likely to be less-than-additive.

Nonylphenol and its Ethoxylates – Beyond the Priority Substance List 2 Assessment. P.M. Campbell[1] and F. Huppé[2]. [1]ToxEcology – Environmental Consulting Ltd., Vancouver, BC; and [2]Environment Canada, Environmental Protection Branch, Montreal, QC.

Nonylphenol and its Ethoxylates (NP/NPEs) were placed on the second Priority Substances List and subsequently underwent a detailed risk assessment by Environment Canada and Health Canada. The Priority Substance List Assessment Report concluded that NP/NPEs were harmful to the environment. As a consequence, on 23rd June 2001, the Ministers of the Environment and Health published their final decision recommending that NP/NPEs be added to the List of Toxic Substances in Schedule 1 under the *Canadian Environmental Protection Act, 1999* (CEPA 1999). NP/NPEs require lifecycle management to prevent or minimize their releases to the environment. Under Section 91(1) of CEPA 1999, the Minister of the Environment must propose a regulation or instrument respecting preventative or control actions to manage NP/NPEs no later than June 23rd, 2003. The instrument must then be finalized by December 23rd, 2004.

A Technical and Socio-Economic Assessment of NP/NPEs indicated that a total of ~18,000 tonnes of NP/NPEs were used annually in Canada in 1998/1999. The main sources of environmental releases of NP/NPEs in Canada have been identified and quantified as far as possible. Risk Management Objectives (RMO) have been proposed by Environment Canada for NPE-containing products and for NP/NPEs in Textile Mill Effluents. The proposed Risk Management Instrument is Pollution Prevention Planning. This paper provides an update on the development of the risk management strategy for NP/NPEs in Canada and compares this to risk management strategies in other jurisdictions.

Estrogenicity and Androgenicity in the Miramichi River, New Brunswick. B.K. Burnison[1], S.B. Brown[1], A. Hobby[2], T. Neheli[1], D. Nuttley[1], D.T. Bennie[1], R. McInnis[1], K. Moore[1], G.J. Van Der Kraak[2] and M.R. Servos[1]. [1]Environment Canada, National Water Research Institute, Burlington, ON; and

[2]Department of Zoology, University of Guelph, Guelph, ON.

Endocrine disrupting compounds may be responsible for the declining Atlantic salmon catches in eastern Canada. Samples were taken from the Miramichi River, New Brunswick in an effort to determine the estrogenicity/androgenicity of the river water and possible major sources, such as pulp mill and treated municipal wastewater effluents. Potential chemical components present in the Miramichi samples were concentrated on SPE columns and subsequently fractionated by HPLC. The estrogenicity of the fractions was routinely measured by the yeast estrogen screen (YES) bioassay and occasionally by an estradiol-EIA procedure. Androgenicity was measured by a testosterone-EIA procedure or by a testosterone receptor-binding assay. Sewage treatment plants (STP) were the main sources of detected estrogenic compounds while the pulp mill contributed some androgenicity and a lower level of estrogenicity. A very polar estrogenic compound was noted in the STPs and was tentatively identified as 16-hydroxyestrone. Estrogenicity was present in the Miramichi River samples several kilometres downstream of the STP outfalls.

Morphological and Physiological Expression of Imposex and Intersex in Five Marine Snails. D. Sotornik[1], L. Viglino[2], E. Pelletier[2] and L.E.J. Lee[1]. [1]Department of Biology, Wilfrid Laurier University, Waterloo, ON; and [2]Institut des sciences de la mer de Rimouski (ISMER), Université du Québec à Rimouski, QC.

The organometallic compound tributyltin (TBT), a common antifouling agent, has been shown to cause endocrine disturbances at very low concentrations in numerous aquatic species. Reports around the globe with various marine molluscs have shown male characteristics appearing in female specimens as a result of exposure to TBT in the wild as well as in laboratory settings. This phenomena known as imposex (imposition of penis and vas deferens over female organs) and intersex (growth of male organs in females) has been described in several prosobranch gastropods. However, the morphological expression of either forms of endocrine disturbance has not been described in detail. Thus in the present study, the physical appearance of imposex and intersex in five marine gastropods abundant in Canadian waters: *Nucella lapillus, N. lamellosa, Buccinum undatum, Illyanassa obsoleta and Littorina littorea,* are presented along with immunological and cardiovascular disturbances noted in some of these snails. Evaluation for imposex and intersex were performed in specimens collected from various sites along the eastern and western Canadian shores. Laboratory exposures *in vivo* and *in vitro* to TBT were also performed with *N. lapillus, I. obsoleta* and *L. littorea*. These findings contribute to better understanding the mechanisms of TBT action and provide clear morphologic images for assessing imposex and intersex in snails.

The Role of Aqueous Bioavailability in the Bioconcentration of Phthalate Esters. D.E. Ratzlaff[1] and F.A.P.C. Gobas$_2$. [1]Department of Biological Sciences,Simon Fraser University, Burnaby, BC; and [2]School of Resource and Environmental Management, Simon Fraser University, Burnaby, BC.

Phthalate esters (PEs) are one of the largest groups of industrially-produced chemicals and consequently are ubiquitous in aquatic environments. Potential toxic effects such as endocrine disruption and carcinogenesis have made PEs the target of hazard assessment by many regulatory agencies. However, PEs are generally hydrophobic and poorly soluble, making it difficult to assess their hazards to aquatic organisms. Measured bioconcentration factors (BCFs) are often much lower than theoretical BCFs, possibly as a result of low bioavailability. This study reports a bioconcentration experiment focusing on the bioavailability of PEs in water. We measured the freely dissolved fraction of PEs using octadecyl (C-18) solid-phase extracts of filtered water. We report BCFs for a variety of PEs and discuss the role of bioavailability with reference to a three-phase sorption model that describes partitioning of PE among the freely-dissolved phase and dissolved and particulate organic matter.

Proposed Assessment Approach for Incorporating the Endocrine Disrupting Potential of Substances into the New Substances Program Under the *Canadian Environmental Protection Act (CEPA)*. N.D. Domey, R.F. Lawuyi and A.J. Atkinson. Environment Canada, New Substances Branch, Hull, QC.

The *Canadian Environmental Protection Act (CEPA)* requires that all new substances be assessed for "toxicity" as defined by the legislation. Proponents of new substances for the Canadian market are required, prior to commencement of manufacture or import, to provide information and test data such as ecotoxicity tests. However, currently there are no ecotoxicity tests in the *New Substances Notification Regulations* that address endocrine disrupting potential. Section 44(4) of CEPA imposes a legal obligation on the Ministers of Environment and Health to conduct research or studies relating to hormone disrupting substances. Currently, Environment Canada is collecting information on such substances and exploring ways it can be incorporated into its regulatory programs. Also, the applicability of structure-activity relationships (SARs) or analogues to identify endocrine disrupting substances (EDS) is being investigated. Each of these areas will be discussed as well as Environment Canada's current approach to addressing endocrine disrupting potential of new substances and the development and validation of international screening tests for EDS.

The Molting Response of Female American Lobsters to Emamectin Benzoate Varies with Reproductive Stage at the Time of Exposure. S.L. Waddy, M.N. Hamilton, L.E. Burridge, S.M. Mercer, D.E. Aiken and K. Haya. Department of Fisheries and Oceans, Biological Station, St. Andrews, NB.

Emamectin benzoate, the active ingredient in Slice®, a fish-feed premix used to control sea lice (*Lepeophtheirus salmonis* and *Caligus* spp.) on farmed salmon (predominantly *Salmo salar*), shortens the 2 year molt cycle of adult female American lobsters (*Homarus americanus*). Egg-bearing lobsters lose their eggs with the cast shell. The molting response is complex and appears to be influenced by both the reproductive stage of the lobsters and seawater temperature at the time of chemical exposure.

Methods

Adult female lobster (300 to 600 g) were obtained from the commercial fishery in Prince Edward Island. They were in intermolt and in either [1] the reproductive phase of their 2 year cycle (pre-spawning), or [2] the molting phase (egg-bearing or resorbing) when exposed to emamectin benzoate on 5 October 2001, 11 March 2002, or 14 May 2002 (see Aiken, 1973; Aiken and Waddy, 1982, for techniques for determining molt and reproductive stages). For each experimental group there was a corresponding control group. Progression through the molt cycle was followed in individual lobsters by examining setal development in the pleopods (Aiken, 1973).

Doses of 0.5 or 1 ìg/g emamectin benzoate were administered by force feeding the lobsters a slurry prepared from salmon feed and medicated with emamectin benzoate (Waddy et al., 2002). Samples of slurry were frozen for later analysis of chemical content.

Lobsters were held communally with shelter in fiberglass tanks under local seawater temperature (varying seasonally from 0° to 15°C) and day length (45°N). They were moved to individual cubicles 1 to 3 weeks prior to molting. Lobsters were fed a varied diet consisting of fresh and frozen invertebrates, fish and seaweed, and a compound feed made from crab meal.

Results and Discussion

When exposed to emamectin benzoate in October 2001, a significant proportion of the lobsters in the molting phase of the cycle (i.e., egg-bearing or resorbing; scheduled to molt in the summer of 2002) responded rapidly and were in premolt, or had molted, by December 2001 (29% vs 0% of reproductive-phase and control lobsters, $p<0.05$). With one exception, molting in the reproductive-phase lobsters did not begin until September 2002 (11 months after chemical exposure, but a year before their normal molting time in 2003).

These sections summarize other results obtained in this study. Egg-bearing and resorbing lobsters exposed to emamectin benzoate in October 2001, March 2002 or May 2002 (scheduled to molt in the summer of 2002). Data pooled for the 3 groups. By 15 June 2002, 56% of the lobsters ($n=96$) exposed to emamectin benzoate had molted or were in premoult, while only 3% of the control lobsters ($n=37$) had reached premolt ($p< 0.05$).

Low temperature in March (3° C) did not prevent the molting response, but did delay the onset of premolt. The mean day of molting in the March and May groups differed by only 8 days (calendar days 197 and 205, respectively), even though the March group was exposed to the chemical 64 days earlier than the May group.

Pre-spawning lobsters treated in October 2001, March 2002 or May 2002 (scheduled to spawn in the summer of 2002 and molt the summer of 2003). Data pooled for the 3 groups. By 15 June 2002, only 1% of the treated lobsters ($n=71$) had reached premolt (vs 0% of the controls, ($p>0.05$), a significantly lower proportion than in the lobsters in the molting phase of the cycle (1% vs 56%, $p<0.05$). By 26 September 2002, 21% of the 71 treated lobsters were in premoult or had molted, a significantly greater proportion than in the control groups (0 of 28) ($p<0.05$).

Conclusions

The molting response of pre-spawning lobsters in the reproductive phase of the 2 year moult-reproductive cycle to emamectin benzoate was significantly different from lobsters in the molting phase of the cycle.

The molting response of lobsters to emamectin benzoate can occur almost immediately (moult has occurred in as few as 48 days) or as late as 11 months after exposure. Both endogenous and exogenous factors appear to contribute to the variation in response (seawater temperature and reproductive stage). Our hypothesis is that premolt is not induced in pre-spawning lobsters until after the endocrine shift that occurs when lobsters enter the molting phase of their cycle following spawning.

The potential risk of this chemical to lobsters foraging under salmon cages is unknown. Work is underway on the feeding behavior of lobsters in response to medicated feed and the effect of ingesting small doses of the chemical over a period of several months. Emamectin benzoate, a GABAergic pesticide, is the only chemical known to induce premolt in a crustacean. This work on the response of lobsters to emamectin benzoate is the first use of induction of premolt as a marker of hormone disruption in crustaceans.

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Determination of Sterol Compounds by Solid Phase Extraction and Gas Chromatography / Mass Spectrometry. M. Chiu. Environment Canada, Pacific Environmental Science Centre, North Vancouver, BC.

Sterols are complex organic alcohols widely distributed in animal lipids. Many of the sterols are endocrine disruptors and can interfere with normal communication between the messenger and the receptor in the cells of both humans and animals. Unfortunately, very subtle effects on the endocrine system can result in unwanted changes in growth, development, reproduction or behaviour that can affect the organism itself, or the next generation. Organic compounds capable of acting as an endocrine disruptor include phytoestrogens, which occur in a variety of plants and fungi and synthetic chemicals, commercially manufactured for a specific purpose or produced as a by-product, such as pesticides, certain polychlorinated biphenyls, dioxins, alkylphenols and synthetic steroids. At Environment Canada's Pacific Environmental Science Centre, a new analytical laboratory method consisting of Solid Phase Extraction and Gas Chromatography/Mass Spectrometry was developed and validated. It has the ability to look for 18 sterol compounds in samples of environment destined effluents such as agricultural run-off, combined municipal storm sewer discharges, non point source discharges, pulp and paper mill waste waters and Sewage Treatment Plant and Waste Water Treatment Plant discharges.

Monitoring Cell Viability in Studies on Gene Expression in Primary Rainbow Trout Hepatocytes. A. Schreer and K. Schirmer. Junior Research Group of Molecular Animal Cell Toxicology, UFZ Centre for Environmental Research Leipzig-Halle, Leipzig, Germany.

We have investigated whether a combination of two fluorescent indicator dyes can be used to monitor the viability of primary rainbow trout (*Oncorhynchus mykiss*) hepatocytes in order to support gene expression analyses upon estradiol exposure. The two fluorescent indicator dyes were alamar Blue, which indicates metabolic activity of a cell, and 5-carboxyfluorescein diacetate acetoxymethyl ester (CFDA-AM), which is an indirect measure of cell membrane integrity. Both dyes can be applied conveniently in multi-well plates without sacrificing the cells. As indicated by both alamar Blue and CFDA-AM, estradiol led to a dose-dependent impairment of cell viability but with alamar Blue, impairment could be detected more sensitively. Concentrations as low as 10^{-8}M estradiol revealed a decrease of conversion of alamar Blue into its fluorescent product. Cytotoxicity caused by estradiol did not appear to be due to a specific regulation of genes as it occurred rapidly and did not change over the course of the 5 day exposures. If the fluorescent dyes can be confirmed to not alter gene expression themselves (using differential display reverse transcriptase PCR), they can be applied in the future to monitor cell viability immediately prior to RNA extraction and gene expression analysis. This unique combination has the potential to yield information essential to understanding the relationship between gene expression and cellular function.

Exposure of Tree Swallows (*Tachycineta bicolor*) to Nonylphenol Ethoxylates and other Contaminants at a Wastewater Treatment Plant.

P. Dods[1], E. Birmingham[1], M.G. Ikonomou[2], T. Williams[1] and J.E. Elliott[3]. [1]Department of Biological Sciences, Simon Fraser University, Burnaby BC; [2]Department of Fisheries and Oceans, Institute of Ocean Sciences, Sidney, BC; and [3]Environment Canada, Canadian Wildlife Service, Delta, BC.

Although nonylphenol is known to have estrogenic effects in aquatic biota, little is known regarding the exposure of nonylphenol ethoxylates to insectivorous birds. This two year study examined reproductive success and growth endpoints in Tree Swallows (*Tachycineta bicolor*) potentially exposed to nonylphenol and other contaminants located in sewage lagoons at a wastewater treatment plant. Tree Swallows breeding next to the Iona Wastewater Treatment Plant were potentially exposed to high levels of nonylphenol ethoxylates through consumption of emergent insects breeding in the sewage lagoons.

The results of the study showed significantly reduced clutch size in the population breeding at Iona Island in both years of study. Fledging success was significantly lower in the potentially contaminated population in the first year of data collection; however, no difference was detected in the second year of study. Nestling growth was similar between sites in both years. Other factors besides contaminant exposure that may influence reproductive success, such as diet composition and frequency of feeding trips, were not found to differ significantly between sites, although tree swallows at Iona Island constructed significantly smaller nests than those at the control site. Levels of nonylphenol ethoxylates detected in sediment, insects and tree swallow liver samples varied between the two years of study. This study reinforces the importance of multiple-year studies when carrying out wildlife toxicology research.

Oil and Gas

Session Co-chairs: D.G. Dixon and I. Johnson

Oil Development in Coastal Waters of British Columbia: A Perspective on Potential Impacts.

J.F. Payne. Department of Fisheries and Oceans, St. John's, NF.

Concern is currently being expressed about the potential effects of oil exploration and development in coastal waters of British Columbia. Development is proceeding in the meantime off the east coast on some of the world's richest fishing grounds such as the Grand Banks where the Terra Nova, Hibernia and White Rose fields are located. What will be the scale and nature of impacts of development whether it is occurring off Newfoundland and Nova Scotia or British Columbia? There is a substantial body of knowledge from field studies in the North Sea, the Gulf of Mexico, California, the Grand Banks and the Scotian Shelf. There are companion laboratory studies including in areas funded by the Program on Energy Research and Development to gain more information of relevance with respect to drill cuttings and production waters.

The majority of information available indicates that impacts will be minimal and pose little or no risk to fisheries and the environment. However there remain knowledge gaps with respect to chronic

toxicity (noted again recently by the NRC) that need to be addressed for reasons of scientific uncertainty or public assurance. The "final" step in addressing uncertainty is to have rigorous monitoring programs that can provide early warning of potential problems. The Canada Newfoundland Offshore Petroleum Board is the regulatory authority for the Grand Banks and all three developments on the Banks are carrying out programs which will provide early warning of any impacts on fish health, fish quality, sediment quality and primary productivity. In perspective, oil development in general is indicated to pose little risk to fisheries and the environment and residual concerns can reasonably be addressed through in-filling of knowledge gaps with respect to chronic toxicity and implementing monitoring programs of an early warning nature.

Risk Assessment of PAH-Contaminated Sediments to Fish Using *in situ* Cages. A.E. Winchester[1], M.E. Bowerman[1], C.H. Marvin[2] and P.V. Hodson[1]. [1]Department of Biology, Queen's University, Kingston, ON; and [2]Environment Canada, National Water Research Institute, Burlington, ON.

The ecological risk of PAH contaminated sediments to fish has historically been assessed through laboratory experiments. These often represent the worst-case scenario in which sediments have been severely disturbed and toxicants resuspended making them bioavailable. Laboratory tests have shown PAH-contaminated sediments are bioavailable, and increase mortality and developmental abnormalities of early life stage fish. The real risk of these sediments cannot be fully understood until complementary *in situ* exposures confirm bioavailability and toxicity under natural conditions. We tested several cage designs for *in-situ* tests with larval fish to address this need. Preliminary field experiments in a coal tar-contaminated area suggest a lower risk to fish from sediment exposure compared with *ex situ* tests. This may be the result of site-specific conditions that limit exposure, such as macrophytes, clean sediment caps, or dilution by water currents. The caging methods may also have contributed to the observed responses of fish, we have adapted our methods to minimize the influence of caging on results.

Sensory Evaluation: Considerations for the Implementation of this Monitoring Tool for Use in Offshore Oil and Oil Spill Environmental Effects Monitoring Programs. S.A. Whiteway. Jacques Whitford Environment Ltd., St. John's, NF.

Adverse effects on the odour, flavour or taste of fisheries resources after contamination by chemical releases or "taint" are of increasing concern to regulatory agencies, corporations and their insurers. A heightened environmental and food safety awareness, multiple stakeholder usage as well as the social and economic consequences of contamination of fisheries resources have resulted in the routine use of sensory evaluation as a monitoring tool for industries that have discharges containing chemicals that have or may have the potential to cause taint to the resource. Historically, tainting complaints and even the perception of taint have been associated with the pulp and paper industry, oil and gas industry, shipping industry and oil spill incidents. There are a variety of sensory methods that can be used and the choice of the actual tool(s) used to assess for taint must take into consideration a variety of factors such as the study objective, the facilities, sample handling, sample preparation, sample presentation, panel composition and the type of panel including assessor selection, panel size, assessor training, and assessor motivation as well as other factors that influence the outcome of taint assessments. Prior to conducting sensory evaluation of any aquatic resources for taint, it is important to have a clear understanding of the objective of the study. Once the study objective is defined, the choice of the appropriate tools (sensory evaluation test and panel type) must be determined. The choice of the tools and the aquatic resource that is assessed will vary from program to program and may extremely different yet ultimate answer the same question. Has the aquatic resource been tainted?

Evidence for the Bioavailability of PAH from Oiled Beach Sediments *in situ*. P.V. Hodson[1], T. Cross[1], A. Ewert[1], S. Zambon[1] and K. Lee[2]. [1]School of Environmental Studies, Queen's University, Kingston, ON; and [2]Department of Fisheries and Oceans, Bedford Institute of Oceanography, Dartmouth, NS.

We measured the bioavailability to fish of sediment PAH from experimentally-oiled freshwater and marine beaches to indicate whether phytoremediation can reduce the exposure and risk of fish to toxic components of oil. Bioavailability was assessed by laboratory bioassays of the activity of cytochrome P450 (CYP1A) enzymes in trout exposed to 500 g of sediments in 10 L of water. CYP1A enzymes, specifically ethoxyresorufin-*O*-deethylase (EROD), increase in activity following the uptake of PAH such as benzo(a)pyrene due to the activation of the cytochrome P450 gene. This bioassay demonstrated that PAH were still bioavailable to fish up to 17 months post-oiling, but that the extent of exposure decreased in parallel to total PAH analyses of sediments. The laboratory tests represent a "worst-case" scenario, in that sediments are disturbed and mixed. Recent experiments with tanks installed directly over beach sediments without disturbing the surface demonstrated that PAH were still taken up by fish *in situ*, 12 months post-oiling, although to a much lesser extent than in the lab. These tests provide useful tools for demonstrating the benefits of phytoremediation in reducing the exposure of fish to PAH.

Chronic Toxicity of Oiled-Sediments to Japanese Medaka. Y. Kiparissis[1], C. Ho[1], J. Reynolds[1], N. Henry[1], P.V. Hodson[1] and K. Lee[2]. [1]School of Environmental Studies, Queen's University, Kingston, ON; and [2]Department of Fisheries and Oceans, Bedford Institute of Oceanography, Dartmouth, NS.

We assessed whether components of oil are toxic to Japanese medaka (*Oryzias latipes*), and if toxicity decreases with aging of oil contaminated sediments. Sediments were collected from an intertidal region in Ste. Croix, Quebec at 6 and 12 months after application of weathered medium MESA oil to experimental plots. Medaka larvae were exposed for 90 days to these sediments. Exposure to 6 months post-application oiled sediment resulted in mortality, reduced growth, fin deformities, liver pathology and reproductive abnormalities. In contrast, oiled sediments collected 12 months post-application were not lethal to medaka and there was a marked reduction in the prevalence of fin deformities and liver pathology. However, growth was equally impaired and the incidence of reproductive abnormalities was similar. These findings indicated that oiled sediments are chronically toxic to fish, and that degradation of oil constituents due to weathering over time may reduce the severity of some toxic effects. However, despite weathering there was little change in effects on other end-points, such as reproduction and growth.

Semipermeable Membrane Devices Concentrate Mixed Function Oxygenase Inducers from Oil Sands and Refinery Wastewaters. J.L. Parrott and L.M. Hewitt. Environment Canada, National Water Research Institute, Burlington, ON.

We performed this study as part of a larger research project that examined fish health in the

Athabasca River in the areas of oil sands. To assess the potential effects of natural and anthropogenic oil sands exposure on liver mixed function oxygenase (MFO) enzymes in fish, we used semipermeable membrane devices (SPMDs) to concentrate bioavailable compounds that may cause MFO induction. Extracts of SPMDs deployed for two weeks on the Steepbank River and in oil refinery wastewater and intake ponds (Athabasca River water) were tested for ability to induce ethoxyresorufin-*O*-deethylase (EROD) activity in *Poeciliopsis lucida* hepatoma cells (PLHC-1), a cell line derived from a liver cancer of a small fish (top minnow).

Semipermeable membrane devices (SPMDs) concentrated MFO-inducing compounds from the Athabasca River and from the refinery wastewater pond. Wastewater pond SPMDs contained very potent EROD inducers in fish liver cells. There were some EROD inducers in SPMDs from the Athabasca River (refinery intake water pond), but these were 1/100th as potent as those in the refinery wastewater pond water (that discharges to the Athabasca River). Preliminary assessment of these same SPMD extracts showed that there were differences in the characteristics of MFO inducers from the refinery wastewater, compared to natural inducers from the oil sands in the Athabasca and Steepbank Rivers. The most potent MFO-inducing compounds from refinery wastewater had log Kows less than 5. In contrast EROD inducers in SPMDs from the Athabasca River water (refinery intake water pond) wastewater and from natural oil sands exposure (Steepbank River) had log Kows of 5 and greater. SPMDs from several sites on the Steepbank R. contained MFO inducers, and the pattern of induction paralleled the MFO induction seen in wild fish: highest MFO inducers were seen in the Steepbank River in the area of the mine (surface anthropogenic disturbance), with less potent inducers in the area of the natural oil sands (undisturbed), and very few inducers in the Steepbank River outside of the oil sands formation.

HPLC Methods for Identification and Analysis of Polycyclic Aromatic Hydrocarbon Metabolites in Fish Tissue. R.S. Brown[1,2], S.P. Tabash[1], P V. Hodson[2] and M.G. Ikonomou[3]. [1]Department of Chemistry, Queen's University, Kingston, ON; [2]School of Environmental Studies, Queen's University, Kingston, ON; and [3]Department of Fisheries and Oceans, Institute of Ocean Sciences, Sidney, BC.

Polycyclic Aromatic Hydrocarbons (PAHs) are well characterized environmental pollutants, but less is known about the alkylated PAH compounds, even though the latter form the major PAH fraction in many sources such as oils. Initial studies have indicated that alkylPAH are significantly more toxic, and we have recently begun developing methods for the analysis and identification of metabolites of alkyl-substituted PAHs. Postmitochondrial supernatant (S9 fractions) from β-naphthoflavone-induced liver tissue was used to generate Phase I metabolites from a series of alkylphenanthrene compounds.

HPLC methods were used to separate metabolites and parent compound from other constituents in bile and liver homogenate, with detection by fluorescence, photodiode array, and mass spectrometry (MS). The metabolism of was dominated by oxidation of the alkyl-carbons on the molecule, including multiple oxidation sites when multiple alkyl-carbons were present, perhaps accounting for dramatic differences in toxicity between these and 'regular' PAH compounds. Phase II metabolites were generated by exposing rainbow trout to the same alkylphenanthrenes, and then isolating and analysing the bile. Glucuronide conjugates of the alkylphenanthrenes were detected, including multiple glucuronides when multiple oxidation was suspected. Further studies involving mass spectrometry to confirm the identity of metabolites and MS/MS to elucidate their structure will be discussed.

The Use of Stable Isotopes to Trace Oil Sands Constituents. A.J. Farwell, V. Nero and D.G. Dixon. Department of Biology, University of Waterloo, Waterloo, ON.

The Athabasca oil sands deposit is the largest of four deposits in northern Alberta, Canada. Mining operations in the region continue to grow as demand for oil increases and new mining technologies are developed. There is a need to better understand the potential effects of oil sands constituents on aquatic ecosystems however defining exposure may be difficult. The objective of this study is to examine the cycling of oil sands constituents in aquatic systems that differ in the level of exposure.

Benthic invertebrates were collected from test pits at Syncrude Canada Ltd. which are similar in dimension (0.16 ha) and constructed in the same year (1989) but differ in the quantity of process-affected water and/or mature fine tailings (MFT) containing residual bitumen. Benthic invertebrates, particularly dragonflies and damselflies, showed trends of ^{13}C depletion and ^{15}N enrichment in pits with increased levels of process-affected water and/or MFT. The depletion of ^{13}C may indicate the assimilation and incorporation of oil sands constituents into the benthic foodweb. Test pits with high turbidity, showed the greatest ^{13}C depletion (~ -27‰), suggesting that microbial degradation of oil sands constituents vs. photosynthetic production may be important in this system. All benthic invertebrate groups (chironomids, amphipods, dragonflies and damselflies) showed an incremental enrichment of ^{15}N from the control pit to the pit with the highest levels of MFT.

Effect of Petroleum Hydrocarbons and Oil Spill Dispersants on Immune Responses in Mussels. D. Hamoutene[1], J.F. Payne[1], A. Rahimtula[2], B. French[3] and K. Lee[4]. [1]Department of Fisheries and Oceans, Science Branch, St. John's, NF; [2]Department of Biochemistry, Memorial University of Newfoundland, St John's, NF; [3]Oceans Ltd., St. John's, NF; and [4]Department of Fisheries and Oceans, Bedford Institute of Oceanography, Dartmouth, NS.

A number of studies have linked anthropogenic stress with an increased incidence of disease in shellfish. We have investigated the effect of water soluble fractions (WSF) of diesel oil and emulsions of an oil spill dispersant (Corexit 9527) on cellular immune responses in mussels (*Mytilus sp.*). Such sublethal effect studies will allow a better understanding of the risks of oil spills on bivalve populations. A secondary objective of this study was the introduction of the cytoskeleton labelling test in cellular response investigation.

Different immune responses were investigated in hemocytes including: number, capacity to phagocytose zymosan particles, adhere to surfaces and maintain cytoskeleton integrity. Studies were carried out *in vitro* by preincubating hemocytes with diesel WSF or Corexit 9527 emulsions prior to measuring phagocytosis and cytoskeleton integrity. *In vivo* immune responses were also investigated in two ways, first by exposing animals to WSF or Corexit emulsions to establish dose-response relationships; secondly, by injecting animals with zymosan particles and measuring phagocytosis and cell numbers in the same mussels before and after exposure to WSF or Corexit emulsions. Overall, the data indicate an effect of dispersant and petroleum hydrocarbons on some aspects of hemocytes immune response. Since dispersant concentrations responsible for alterations of mussel immune functions were higher than 100 mg/L, we can conclude that Corexit 9527 is not likely to affect cellular mussel functions if used in operational doses during oil spills. On the other hand, the effect of diesel WSF could represent a threat to mussel immune responses, the "effective" doses obtained in this study (8.22, 11 mg/L) being comparable or higher than those expected in the water column during treatment of an oil spill. This study also shows the potential use of cytoskeleton labelling test in mussel immunity assessment.

Fish Gill Responses to Pollutants from Oil Sands Mining-Associated Waters. L.E.J. Lee[1], V. Nero[2], S. Willfang[1], M.P. Lamb[1], A.J. Farwell[2] and D.G. Dixon[2]. Department of Biology, [1]Wilfrid Laurier University, Waterloo, ON; and [2]Department of Biology, University of Waterloo, Waterloo, ON.

Large amounts of tailings water are associated with the extraction of petrochemicals from the oil sands of Athabasca, Alberta. High salinity and naphthenic acids (NAs) within these waters have been implicated in toxicity to aquatic biota along with polycyclic aromatic hydrocarbons (PAHs). Whereas the toxicity of PAHs have been widely addressed, the effects of sulfate/chloride salinity and NAs remains to be elucidated. NAs are complex mixtures of saturated carboxylic acids whose exact chemical composition is not known.

In vivo and *in vitro* evaluation of crude and individual NAs and salts, along with *in situ* exposures of fish to reclamation ponds were performed in order to investigate effects on gills, a highly susceptible tissue to contaminants. Yellow perch exposed for 3 weeks in reclamation ponds (Pit 3 and Pit 5) showed higher incidence of gill pathological changes than control fish held in Mildred Lake, a reservoir lake whose waters are diverted for use in oil sands extraction. *In vivo* exposure to sulfate/chloride salts or to abietic acid, a tri-cyclic carboxylic acid, resulted also in notable gill histopathological changes in fathead minnows. *In vitro* exposures of individual salts, commercial NAs or crude NA extracts from the reclamation ponds, caused changes in membrane integrity, mitochondrial function, lysosomal activity and general morphology in a rainbow trout derived gill cell line (RTgill-W1). This approach provides a suite of tests for in-depth and rapid analysis of tailings water toxicity and contributes to environmental risk assessment. (Supported by TSRI and CWN).

Does Chemically Dispersing Crude Oil Increase the Exposure of Fish to Polynuclear Aromatic Hydrocarbons (PAH)? S.D. Ramachandran[1], C.W. Khan[1], K. Lee[2] and P.V. Hodson[1]. [1]School of Environmental Studies, and Department of Biology Department, Queen's University, Kingston, ON; and [2]Department of Fisheries and Oceans, Bedford Institute of Oceanography, Dartmouth, NS.

The use of oil spill dispersants is a controversial countermeasure in the effort to minimize the impact of oil pollution. The risk of ecological effects will depend on whether oil dispersion increases or decreases the exposure of aquatic species to the toxic components of oil. To evaluate whether fish will be exposed to more PAH in dispersed oil relative to equivalent amounts of water accommodated fraction, we measured CYP1A induction and bile metabolites of polycyclic aromatic hydrocarbons (PAH) in trout exposed to dispersant (Corexit 9500), water accommodated fractions (WAF) and chemically enhanced water accommodated fraction (CEWAF) of three crude oils. Medaka eggs were also exposed to dispersant Corexit 9500, WAF and CEWAF. Total petroleum hydrocarbon (TPH) and polycyclic aromatic hydrocarbon concentrations in the test medium were determined to relate observed CYP1A induction in trout and ascites in medaka embryo to dissolved fractions of the crude oil. Preliminary results showed 10 fold higher induction in fish exposed to CEWAF as opposed to (WAF). Trial PAH measurements have shown higher concentrations of alkylated PAH's, mostly methylated naphthalene, in the dispersed oil in water as compared to the water accommodated fraction. Medaka embryos showed the following trend with increasing hydrocarbon concentrations, early hatch > mild edema > severe edema > mortality.

Gill Histopathology of Goldfish Exposed to Oil Sands Processed-Waters. V. Nero[1], A. Lister[2], A.J. Farwell[1], L.E.J. Lee[3], G.J. Van Der Kraak[2] and D.G. Dixon[1]. [1]Department of Biology, University of Waterloo, Waterloo, ON [2]Department of Zoology, University of Guelph, Guelph, ON; and [3]Department of Biology, Wilfrid Laurier University, Waterloo, ON.

The extraction of oil from the Athabasca oil-sands (Alberta, Canada), produces vast volumes of processed wastewater consisting of naphthenic acids (NAs), polycyclic aromatic hydrocarbons (PAHs) and high salinity. This wastewater has been classified as acutely toxic to aquatic biota due to the naturally occurring NAs. Goldfish, *Carassius auratus*, were placed within enclosures in three different experimental ponds (Pit 1, 3, and 5) to test for biological effects of aquatic reclamation alternatives for process-affected waters. Pit 1 represented the reference (control) pond while Pits 3 and 5 contain known varying chemical makeup. Following 19 days of exposure, severe gill histopathological changes were noted in fish kept in Pit 5, which contains elevated levels of Nas and salinity. Morphological anomalies included extensive epithelial hyperplasia leading to severe lamellar fusion, chloride cell hyperplasia, and lamellar oedema. Rather than reflecting direct toxic action, these alterations have been interpreted as defense responses of the fish. In addition to the observations above, more severe gill alterations that may reflect the direct deleterious effects of oil sands processed-water were observed. Necrosis and degenerative changes leading to desquamation of the epithelial surfaces of the gill most often occurred in exposure of goldfish to Pit 5 water. These observations are compared to the effects seen during a free-range exposure of a native fish species, yellow perch (*Perca flavescens*), at the same sites and exposure time.

Aquaculture

Session Co-chairs: K. Haya and R. Endris

Assessment of the Environmental Risks Associated with Chemical Use in Finfish Aquaculture. W.R. Ernst, W.R. Parker, K.G. Doe, P.M. Jackman, G. Julien and J. Aube. Environment Canada, Atlantic Region.

The use of chemicals in aquaculture, particularly finfish aquaculture, has raised concerns in the regulatory and public communities. The emerging environmental fate and effects database, indicates that the use of some of the sealice control products such as cypermethrin, which has been proposed for sealice control in Canada and which has been used operationally in the US, have the potential for widespread impacts because of the fact that chemicals used in aquaculture are generally released freely to the environment. Recent inventory work by Environment Canada has revealed that there are a wide variety of chemicals used in aquaculture however, but their impacts remain uncertain.

Concerns about possible chemical residues in the sediments that accumulate under salmon net pens has prompted Environment Canada to participate in a recent series of studies to measure contaminants in sediments. Chemicals of concern have included: metals, PCB, DDT, pesticides, and PAH's. The results have indicate that Cu and Zn were elevated at some salmon farm sites. Sediment toxicity tests on undercage sediments have also been conducted to assess the potential impact of contaminants. Although sediments were found to have varying levels of toxicity, that toxicity was not directly related to contaminant concentration, but was related to ammonia and sulfide contents of the sediments. Due to the intense nature of aquaculture in Canada, particularly the Atlantic coast, and the potential for conflicts with other marine resource users, additional assessments of the aquatic risk of chemical use in aquaculture are needed.

Effects of Pesticides Used in Aquaculture on American Lobsters. L.E. Burridge[1], S.L. Waddy[1], K. Haya[1], N. Hamilton[1], S.M. Mercer[1] and R. Endris[2]. [1]Department of Fisheries and Oceans, Biological Station, St. Andrews NB; and [2]Schering-Plough Animal Health, Union, NJ.

The use of pesticides to combat infestations of parasitic sea lice is a normal part of salmon aquaculture operations in eastern Canada. Pesticide use has given rise to concerns regarding their potential effect on non-target crustaceans. Salmosan® (47.5% w/w azamethiphos) is the only pesticide registered for use against sea lice in Canada. Treatment is a 30 or 60 minute bath in 100 µg/L. An in-feed additive, Slice® (0.2% emamectin benzoate (EB)), is available under an emergency drug release from Health Canada. The recommended treatment dose is 50 µg (a.i.)/kg (fish) per day for 7 consecutive days. Concentrations of EB in fish food during operational treatments can be up to 25 mg/kg but commonly range from 5 to 10 mg/kg. Lethality tests were conducted with azamethiphos and adult female lobsters over a two year period spanning their biennial spawning/molting cycle. The 48 hour LC50 was found to range from 0.61 µg/L in August to 3.24 µg/L in April. There were significant differences with season (ANOVA) and multiple comparisons indicate that lobsters are less sensitive to azamethiphos in February and April than in August ($p<0.05$). Data suggest time of exposure may play an important role in assessing the risk of this pesticide to lobster. A range of concentrations of EB was incorporated into commercial salmon food and provided to adult lobsters over a 7 day period. Lobster did not consume sufficient EB-treated food to result in 100% mortality in any group (maximum concentration = 1280 mg/kg of food). The estimated LC50 of EB is 735 mg/kg of food. Lobsters have also been force-fed a slurry of medicated food at nominal doses ranging from 0.25 to 4 mg/kg (lobster weight). No concentration resulted in 100% mortality in the treated groups. In May/June the estimated LD50 is 1.23 mg/kg. Lobsters appear to be less sensitive to EB in October/November, when doses as high as 4 mg/kg resulted in <50% mortality.

Is the Elevated Copper Frequently Observed in Sediments Near Salmon Aquaculture Sites Toxic to Marine Amphipods? W.R. Parker[1], K.G. Doe[2] and P.M. Jackman[2]. [1]Environmental Protection Branch, Environment Canada, Fredericton, NB; and [2]Environmental Conservation Branch, Environment Canada, Moncton, NB.

Several surveys in Canada and Europe have determined that the concentration of Cu in the sediments around salmon aquaculture sites can be elevated above background concentrations. Cu concentrations in these sediments often exceed the CCME Interim Sediment Quality Guideline (ISQG) for copper and occasionally exceed the probable effects level (PEL). The most probable source of the Cu is the copper-based protective coatings that are applied to the nets used in salmon pen construction. These coatings are intended to eliminate or reduce the fouling of the nets by marine invertebrates and algae. For this study, sediment toxicity tests with the marine amphipod, *Eohaustorius estuarius* were conducted on sediment samples collected directly under salmon net pens and on sediment samples from a couple of harbours in the local area that had frequent fishing or recreational boat traffic. In addition, samples of clean sediment were spiked with copper-based anti-fouling coatings to concentrations that were equal to the ISQG, equal to the PEL, and concentrations that exceeded the PEL levels by 5 fold and these spiked sediments were also tested to amphipod toxicity. The paper will present the findings of these tests.

The Environmental Response to Copper and Zinc Introduced into Marine Environments Near Salmon Farms in the Pacific Northwest. K.M. Brooks. Aquatic Environmental Sciences, Port Townsend, WA.

Copper oxide antifouling treatments are increasingly being used to reduce or prevent fouling on aquaculture containment nets and Zn is a micronutrient added to salmon feeds. Elevated sediment concentrations of these metals have been observed near some salmon farms in British Columbia since 1996. In some cases, the concentration of Zn exceeds several environmental affects benchmarks and or regulatory criteria. Metal binding by sulfides in the vicinity of salmon farms will be shown to effectively detoxify these metals during production. Sediment Zn concentrations were observed to decline to background during chemical and biological remediation during a long-term benthic effects study at Stolt Seafarm's Arrow Pass Atlantic salmon farm in British Columbia. Laboratory leaching studies on copper oxide treated salmon farm containment nets have produced a spreadsheet model for predicting water column concentrations of Cu as a function of time following net immersion and netpen configuration. Recommendations for managing sediment concentrations of Cu and Zn near salmon farms will be made based on the data presented.

Organic Contaminants in Feed Used by, and Sediments from Under Salmon Aquaculture Sites of Eastern Canada. J. Hellou[1], S. Steller[1], K. Haya[2] and L.E. Burridge[2]. [1]Department of Fisheries and Oceans, Bedford Institute of Oceanography, Halifax, NS; and [2]Department of Fisheries and Oceans, Biological Station, St. Andrews, NB.

Food pellets used in salmon aquaculture can vary in size from 1 to 10 mm and contain an increasing level of lipids (20 to 30%), with ingredients deriving from offshore fisheries and land based products. Salmon aquaculture is prospering in New Brunswick and raises questions about the fate of unintentionally used lipophilic contaminants and their deposition around cages. Commercial food pellets, fish oil used as an ingredient in feed, and sediments collected around cages in 1998 and 1999 were analysed for three groups of organic contaminants.

Alkylated naphthalenes were detected in food pellets, fish oil and sediments, while other polycyclic aromatic hydrocarbons were detected at variable levels in sediments only. Some PCB congeners and *p,p'*-DDE were also detected at low levels in all samples. Trends were observed during the first year of sediment sampling, with up to 6 to 8 times higher organic carbon content and levels of PCBs and *p,p'*-DDE below the cages relative to 25 meters away. During 1999 vs 1998 and therefore longer use of the sites, levels of *p,p'*-DDE and PCBs were somewhat reduced under the cages but detected up to 100 meters away from the cages. Analyses of samples collected in 2000 are still ongoing. Level of contaminants determined in food pellets and sediments will be compared to those reported for food items and sediments from around the world.

Linking the Fate of Aquaculture-Associated Chemicals to Organic Matter Cycling in Aquatic Systems. A.M.H. deBruyn and F.A.P.C. Gobas. School of Resource and Environmental Management, Simon Fraser University, Burnaby, BC.

Organic chemicals which have low solubility in water become associated with living and dead organic matter (OM) in aquatic environments. Current models and assessment methods view this OM as an inert matrix within which chemicals achieve an equilibrium distribution according to their thermodynamic properties. It is well known, however, that OM in aquatic systems is a dynamic entity that cycles on time scales much shorter than those required for many chemical distribution processes to approach equilibrium. Here, we use a dynamic model of simultaneous chemical partitioning and OM diagenesis to show how the fate of organic chemicals associated with salmon aquaculture is strongly linked to OM cycling in the receiving water environment. The model predicts that low-level OM enrichment of the sediment will promote remineralization, inflating chemical fugacities and increasing the risk of contamination to the benthic biota. High rates of OM sedimentation will promote sediment burial, keep chemical fugacities in the sediment low, and reduce contamination risk to benthic biota.

Development of Sulfide Tolerance Benchmarks for 85 Macrobenthic Species Found in Pacific Northwest Marine Environments. K.M. Brooks. Aquatic Environmental Sciences, Port Townsend, WA.

Sediment free sulfide and macrobenthic community data collected in 255 0.1 m^2 van Veen grab samples has been used to define a Maximum sulfide tolerated (MST), Highest Effects Level (HEL), and Maximum Enhancement Level (MEL) for 85 common macrobenthic taxa found in the Pacific Northwest. Significant differences were observed in con-generic taxa such as *Macoma nasuta*, which tolerates sediment sulfide concentrations as high as 3,500 micromoles sulfide, whereas *Macoma inquinata* was not found in sediments containing greater than 300 micromoles. The importance of understanding sulfide concentrations found in natural environments and in choosing laboratory bioassay test animals will be discussed and compared with an extensive polycyclic aromatic hydrocarbon and macrobenthic database collected during Phase II of the Sooke Basin Creosote Evaluation study completed by Environment Canada in 2001.

Relationship Between Sediment Physicochemical Variables and Macrobenthic Communities Near Atlantic Salmon Farms in the Pacific Northwest. K.M. Brooks. Aquatic Environmental Sciences, Port Townsend, WA.

Intensive monitoring at 34 salmon farms in British Columbia and 10 farms in Washington State since 1991 has revealed consistent relationships between sediment chemistry and macrobenthic community response. Chemical and biological effects in sediments were highly correlated with free sulfides and redox potential and extended to distances of 205 meters from the cages. Species diversity declined with increasing sulfides without a lower no-effects concentration. The abundance of macrofauna was enhanced by at least eight opportunistic species of arthropods and annelids between 200 and 15,000 micromoles free sulfide. Sediments were generally, but not always, depauperate at sulfide concentrations greater than 6,000 micromoles.

These studies have clearly demonstrated the usefulness of sediment free sulfides for predicting the biological response to benthic enrichment. The inappropriateness of using total volatile solids or total organic carbon as a sole physicochemical surrogate for benthic community analysis will be described. Sediment physicochemical data collected over a period of several years at numerous farms clearly demonstrates the relationship between farmed salmon production and sediment chemical remediation. These studies have revealed several areas of research needed to improve siting and management of intensive finfish culture.

Antibiotic Resistant Bacteria in Sediments Collected near Salmon Aquaculture Sites in New Brunswick, Canada. K. Haya[1], B.T. Hargrave[2], S. Armstrong[3], F. Friars[3], K. MacKiegan[1] and L. Doucette[2]. [1]Department of Fisheries and Oceans, Biological Station, St. Andrews, NB; [2]Department of Fisheries and Oceans, Marine Environmental Sciences Division, Dartmouth, NS; and [3]Department of Biology, Dalhousie University, Halifax, NS.

Feed medicated with antibiotics is used to treat bacterial infections of cultured salmon. In New Brunswick the antibiotic of choice is oxytetracyline (OTC). OTC may accumulate in sediments from sedimentation of excess food pellets and fish wastes near farm sites and may lead to the selection, development and growth of oxytetracycline resistant populations of bacteria. Resistance to OTC was determined in aerobic bacteria cultured from surface sediments collected near and distant from salmon aquaculture sites in the Fundy Isles Region of New Brunswick. All sediments (n=52) collected within 100 meters of active salmon aquaculture sites had OTC resistance above the threshold for establishing resistance (minimum inhibitory concentration (MIC) >25 µg OTC per ml). Controls consisting of a culture of *Areomonas salmonicida* and bacteria cultured from sediments collected >100 meters away from the salmon aquaculture sites, from intertidal areas in Letang Inlet and Passamaquoddy Bay and from a Nova Scotia embayment (n=7) showed no resistance to OTC (MIC <5 µg OTC per ml). An analysis of the 16S rRNA gene from some of these isolates suggests that the species isolated belong to the genus *Psychrobacter.* The quantitative analysis of sediment extracts for OTC by LC-MS is in progress.

Investigation of Acute Sublethal Effects of Rotenone on the Routine Oxygen Uptake of Juvenile Rainbow Trout (*Oncorhynchus mykiss*). D. Jayaweera. Department of Biological Sciences, Simon Fraser University, Burnaby, BC.

The sublethal toxicity on the routine metabolic rate of the well-known piscicide rotenone was tested on juvenile rainbow trout (*Oncorhynchus mykiss*). The hypothesis of this study was that the exposure to sublethal concentrations of rotenone would cause respiratory stress in fish, given its known inhibitory effects on the mitochondrial electron transport chain. To test this hypothesis, experiments were performed in a flow-through respirometer system that measured oxygen uptake of fish for 48 hour prior to and 48 hour during rotenone exposure at 12 to 15°C. An acute lethality study showed that the 96-h LC50 of rotenone for rainbow trout was 13.3 µg/L under present experimental conditions.

We tested four concentrations of rotenone up to 37% of the 96 hour LC50 value. The routine oxygen uptake for 0.5, 1.8, 2.5 and 5.0 µg/L rotenone was 200, 195±26.4, 180±15.8 and 180±42.2 mg O_2/kg/h, respectively. The rate of routine oxygen uptake of rotenone-exposed fish was not significantly different from the control which was 200±26.4 mg O_2/kg/h. Based on the findings of this study, I concluded that the routine oxygen uptake of juvenile rainbow trout was not significantly affected by rotenone at concentrations approaching half of the 96 hour LC50 value despite rotenone's known inhibitory effects on the mitochondrial electron transport chain.

Toxicity of Emamectin Benzoate in Fish Feed to Adults of the Spot Prawn and Dungeness Crab. M.R. Linssen[1], G.C. van Aggelen[1] and R. Endris[2]. [1]Environment Canada, Pacific Environmental Science Centre, North Vancouver, BC; and [2]Schering-Plough Animal Health Corp., Union, NJ.

Sea lice infestations of net-penned Atlantic salmon can result in significant economic losses to the salmon aquaculture industry in Canada. The use of prophylactic chemicals are necessary prescriptions for control of this problem to the aquaculture industry. Their use and application is regulated in order to protect indigenous species and the marine environment in general. Toxicological data for relevant species is critical for the evaluation of potential use in Canada. Environment Canada's Pacific Environmental Science Centre-Toxicology Section, working with the

Commercial Chemicals Section of EC-PYR and the Schering-Plough Animal Health Corporation, conducted toxicological testing on Spot prawns, *Pandalus platyceros*, and Dungeness crab, *Cancer magister*, Studies used the licicide Slice® (active ingredient emamectin benzoate) developed by Schering-Plough Animal Health Corporation. The studies were designed to investigate the potential deleterious effects of the active ingredient in the sea licicide as administered in medicated fish feed to Pacific Northwest aquatic species. Laboratory methods, testing apparatus and results will be presented.

Environmental Impacts of Freshwater Aquaculture in Canada: Towards Science-Based Tools for Sustainable Management. S.S. Dixit[1] and S.L. Walker[2]. [1]Environment Canada, National Guidelines and Standards Office, Hull, QC; and [2]Environment Canada, National Environmental Effects Monitoring Office, Hull, QC.

In August 2000, the Federal Government implemented a 5 year Program for Sustainable Aquaculture in Canada. Environment Canada was given the responsibility to develop applied science tools (i.e., Environmental Effects Monitoring and Environmental Quality Guidelines) and conduct research to assess the effects of aquaculture operations on freshwater ecosystems. Freshwater aquaculture usually involves growing a large number of fish in a small space. As a result, large quantities of waste are generated that have potential environmental consequences.

Environmental impacts of aquaculture on freshwater ecosystems are largely dependent upon the site conditions, the type and size of aquaculture operation, and the nature of the wastes generated and chemicals used at the operation. Potential impacts include: [1] increased loadings of nutrients resulting in eutrophication and deterioration of water quality, [2] increased organic matter impacting sediment quality and benthic communities, [3] releases of therapeutant chemicals, [4] increased abundance of pelagic and bottom dwelling organisms around net cages, [5] escape of farmed fish causing increased competition for habitat and food with wild fish and potential genetic impacts, [6] increased transmission of diseases between wild and farmed populations, and [7] potential loss of habitat, injury, and persecution for wildlife and species at risk. A review of the existing data indicates that Canadian research studies are lacking for freshwater aquaculture. Ongoing environmental monitoring programs lack consistency and *Canadian Environmental Quality Guidelines* are not available for many chemicals used in aquaculture operations. Necessary steps are being taken to identify information gaps, science and research needed for science-based, sustainable management of freshwater aquaculture in Canada.

Sediments

Session Co-chairs: U. Borgmann and K.G. Doe

A Tool for Monitoring Trace Metals in Freshwater Sediments. L. Croisetièr, L. Hare and A. Tessier. INRS-ETE, Université du Québec, Ste-Foy, QC.

Mining and smelting activities have led to large increases in the concentrations of sedimentary trace metals in aquatic and terrestrial ecosystems. Evaluating the risks associated with metals is dependent on estimates of their bioavailability. Measurements of trace metals in animals allow us to use them as biomonitors to quantify metal exposure and, using an appropriate model, estimate bioavailable metal concentrations in water or sediment. To be effective, such models should be based on chemical and biological knowledge about the organism and the metal.

The predatory insect *Sialis* (Megaloptera) has been proposed as a monitoring tool for sedimentary metals. Larvae are tolerant of metals and of extremes of pH as well as being widespread in North American and European lakes. Field experiments allowed us to determine the route of exposure of metals to *Sialis* larvae (food, water or sediment) and the relationships between ambient and insect metal concentrations. Our results suggest that *Sialis* takes up metals (Cd and Pb) mainly from its prey and that metal concentrations in this insect can be related to those in lakes through the use of a model that takes into account competition between hydrogen ions and free metal ions for biological uptake sites. Our data suggest that *Sialis* would be an effective tool for monitoring metals in lakes.

A Comparative Study of the Toxicity and Bioaccumulation of Tributyltin in 6 Species of Freshwater Invertebrates. A.J. Bartlett[1], D.G. Dixon[1], R.J. Maguire[2], S.P. Batchelor[2] and U. Borgmann[2]. [1]Department of Biology, University of Waterloo, Waterloo, ON; and [2]Environment Canada, National Water Research Institute, Burlington, ON.

Although the toxicity of tributyltin (TBT) has been well documented in marine organisms, considerably less is known about the impact of TBT in freshwater environments. This study compares the effects of TBT on six freshwater invertebrates: *Hexagenia limbata*, *Physella gyrina*, *Tubifex tubifex*, *Hyalella azteca*, *Chironomus riparius* and *Daphnia magna*. This selection of species spans a range of life cycles, feeding habits, and burrowing behaviours, all of which may affect toxicity. Young from each species were added to aquariums containing control, low, medium, or high concentrations of TBT-spiked sediment. The addition of each species to the aquariums was staggered in order of decreasing life cycle length.

Survival, growth, reproduction, and bioaccumulation were measured. Survival was affected at the highest concentration of TBT: no *Hexagenia*, *Tubifex*, or *Daphnia* survived, and *Hyalella* and *Physella* numbers were significantly diminished. Growth was not affected by TBT; total mass remained fairly constant regardless of concentration, indicating a compensatory effect of increasing mass with decreasing survival. Reproduction appeared to be affected in *Physella* at the highest concentration, but was highly variable in *Daphnia*, *Hyalella*, and *Tubifex*. The bioassays were ranked in terms of increasing sensitivity: *Chironomus* < *Hyalella* < *Physella* < *Tubifex* = *Daphnia* < *Hexagenia*. Bioaccumulation of TBT was similar in all species, despite the observed differences in toxicity. The sensitivity of the *Hexagenia* test makes it an ideal candidate for future toxicity studies to further characterize the impact of TBT on freshwater environments.

Bioavailability of Sediment-Associated Trace Metals - Does the Test Container Make a Difference? J.C. Evans and D.G. Dixon. Department of Biology, University of Waterloo, Waterloo, ON.

The bioavailability of sediment-associated trace metals is dependent on the geochemical phases with which they are associated. Since the partitioning of metals into these phases depends on both the composition and oxidation state of the sediment, the particular physical conditions under which the sediment is tested may have a profound effect on the results. *Tubifex tubifex* and *Chironomus tentans* were exposed to a mixture of Cd, Cu and Ni (added as iron oxyhydroxide coprecipitates) over a 3 week period. Three types of test container were used: cups containing 160 mL water and 40 mL sediment (4:1 ratio), Imhoff settling cones containing 1000 mL water and 15 mL sediment

(67:1 ratio), and recirculating tanks containing 56 L of water and 28 flow-through cups with 40 mL sediment apiece (for a pooled volume ratio of 50:1). Differences in metal bioavailability (as measured by metal tissue content after gut-purging in fresh sediment), and the final metal concentrations in overlying water, centrifuged pore water and various sediment geochemical phases were measured for the three metals. Results suggest that the tank and cone containers produce similar results with respect to tissue body burdens and porewater metal concentrations, while the more traditional cup containers showed some interesting differences, most notably with respect to reduced Cd and Ni bioavailability to *T. tubifex*.

Nickel Bioavailability and Toxicity to *Hyalella azteca* in Freshwater Sediment. L.E. Doig and K. Liber. Toxicology Centre, University of Saskatchewan, Saskatoon, SK.

Total metal content has been shown to be a poor predictor of bioavailable metal content in sediment. For sediment associated, divalent, cationic metals, the free ion concentration in pore water is thought to represent the main bioavailable fraction. Understanding the partitioning of metals between solution and solid phases is therefore important in determining metal bioavailability and toxicity in sediments. Acid volatile sulfide (AVS) is thought to be the main binding phase governing pore-water metal concentrations (e.g., Ni, Cd, Cu, Pb and Zn) in anaerobic sediments. Those metals in excess of AVS capacity are believed to have the potential to be bioavailable to the associated benthic biota. Currently, we are studying how organic matter (in addition to sulfide) affects nickel bioavailability in freshwater sediments. Artificial freshwater-sediments were constructed so that the organic carbon content varied (1.7 to 10% OC) while other sediment constituents remained constant.

Ten day toxicity tests (using the common benthic amphipod, *Hyalella azteca)* were conducted with Ni spiked over a range of concentrations. Results indicated that total organic matter content of sediment had a significant influence on NI bioavailability to *H. azteca*. Artificial sediments with different amounts of organic matter (OM) displayed a clear decrease in toxicity with increasing OM content at the same total Ni concentration. Results further indicated that toxicity was strongly correlated with pore-water Ni concentration, and that toxicity estimates based on pore-water Ni exposures were comparable to separate toxicity estimates for nickel in water-only tests.

Effects of Water Source on Metal Bioavailability and Toxicity from Field Collected Sediments. M. Nowierski[1,2], D.G. Dixon[1] and U. Borgmann[2]. [1]Department of Biology, University of Waterloo, Waterloo, ON; and [2]Environment Canada, National Water Research Institute, Burlington, ON.

The toxicity of sediments to *Hyalella azteca* is a function of the amount of metal accumulated and not on the concentration of total metals in sediment. Metals responsible for toxicity can be identified by comparing metal bioaccumulation to critical body concentrations. Standard sediment toxicity tests use laboratory water as overlay water. Lake water chemistry (hardness, pH, DOC, etc.), however, is highly variable and is known to affect metal speciation, binding to sediments and toxicity. The release of metals from sediment, and the toxicity of those metals once in solution, could therefore be affected by overlying water chemistry. The objective of this study is to compare metal bioavailability and toxicity in laboratory toxicity tests with field collected sediments using both standard laboratory water and natural lake waters. Two lakes from Sudbury, Ontario and three lakes from Rouyn-Noranda, Quebec were selected as locations for both sediment and water collection based on high metal bioavailability in the sediment and a wide range of lake-water chemistry.

One week bioaccumulation and four week chronic toxicity tests were completed using the amphipod *H. azteca*. A greater reduction in survival and growth was apparent following exposure to sediments with overlying water of lower buffering capacity. Bioaccumulation of Ni from Sudbury sediments neared or exceeded the critical body concentration known to cause toxicity (Ni LBC25 = 298 nmoles/g dwt). Bioaccumulation of Cd from one of the Noranda area sediments neared the LBC25 value (Cd LBC25 = 283 nmoles/g dwt). Mobilization of both Ni and Cd from sediment into overlay water increased with increasing H^+ ion concentration. In terms of metal uptake by the organism, less Ni accumulated in softer waters, while the reverse was true for Cd. The H^+ ion effect predominates for Ni binding, while the Ca^{2+} ion effect predominates for Cd accumulation by the organism.

The Role of Bacterial Versus Sediment Sources in Mediating Metal Bioavailability to the Bivalve *Mytilus trossulus*. J.R. King, L.I. Bendell-Young, M. Moore, D. Crozier and A. Jurgensen. Department of Biological Sciences, Simon Fraser University, Burnaby, BC.

The marine bacterium *Leptothrix discophora* SP-6 is unique in its ability to oxidize soluble forms of Fe and Mn. These metals become sequestered in an electronegative oxide sheath around the bacterial cell membrane and are potential uptake sources to filter-feeding bivalves. Our research examines how metals such as Cd and Pb attach to the SP-6 sheath and become bioavailable to the marine bivalve, *Mytilus trossulus*. SP-6 samples were cultured with various combinations of Mn, Cd and Pb and fed to the bivalve *M. trossolus* using a flow-thru system. In addition, natural sediments high in Mn, Cd and Pb were collected from Boundary Bay, BC, Canada and fed under the same conditions.

Results of metal tissue concentrations obtained with an AA spectrophotometer indicates that Mn uptake by *M. trossolus* was not highly significant for either bacterial or sediment matrices ($F=3.50$, $p>0.004$). When Cd and Pb uptake were examined, significant differences were found to exist amongst treatments. In particular, the Mn sheath on SP-6 appeared to decrease both Cd ($F=33.65$, $p<0.0001$) and Pb ($F=7.39$, $p<0.0001$) uptake when compared to SP-6 treatments without Mn and natural sediments. These results suggest that SP-6 with its Mn sheath acts to decrease potentially toxic metal uptake to *M. trossolus* when soluble forms of Cd and Pb are present under controlled laboratory conditions.

Investigations into Causes of Sediment Toxicity with Bivalve Larvae. H.C. Bailey, J.R. Elphick, A.R. Tang and G.S. Lawrence. EVS Environment Consultants, North Vancouver, BC.

Bivalve larvae are frequently used in sediment quality triads as a measure of toxicity. In many cases, such tests are the most sensitive indicators of adverse effects. Identifying the causes of any effects may become important if a variety of contaminants are present, if it is necessary to establish liability in cases of multiple users or discharges, or if it is desirable to differentiate between natural and anthropogenic causes of effects. Finally, testing artifacts, such as settling particulate material, may also contribute to larval mortalities. This presentation describes a number of techniques that have been successfully used to identify causes of toxicity in the larval bivalve sediment toxicity test and how they can be systematically applied to differentiate between potential sources of toxicity.

Monitoring Toxicological Changes During Biotransformation of Munitions in Sediments: Microbial Community Profiling. B.T. Johnson[1], M. Nipper[2], R.S. Carr[3] and K. Miller[4]. [1]Columbia

Environmental Research Center, USGS, Columbia, MO; [2]Texas A&M University-Corpus Christi, TX; [3]Marine Ecotoxicology Research Station, USGS, Corpus Christi, TX; and [4]Navy Facilities Engineering Service Center, Port Hueneme, CA.

The oceans of the world are common repositories as dumping grounds for military munitions during both acts of war and times of peace. Concurrently to the assessment of changing acute toxicity of two explosives of concern - trinitrophenol (picric acid) and 2,6-dinitrotoluene (2,6-DNT) - spiked in marine sediments (Nipper et al., this meeting), changes in the microbial community responsible for their biotransformation were monitored. Microbial profiles of bacterial diversity of aerobic heterotrophs were obtained using enzyme specific assays: Colilert (Idexx) for total coliforms, SimPlate (Idexx) for total heterotrophs, and EcoPlates (Biolog) for microbial diversity done in different marine sediments (sandy vs. loamy, low vs. high TOC) over a 56 day interval (1, 3, 7, 14, 28 and 56) at 10°C and 20°C under aerobic conditions.

The explosives disappeared faster in the loamy, high TOC sediment at 20°C; HPLC chromatograms suggested that both compounds completely disappeared within 8 to 12 weeks. Most-probable-number assays of total heterotrophic populations during biotransformations clearly reflected significant population changes with an initial 2 to 10 fold decrease in bacterial counts between 1 to 3 days followed by rapid recovery by day 7. Microbial profiling clearly demonstrated measurable qualitative and quantitative changes in both heterotroph activity and bacterial diversity during 2,6-DNT and picric acid biotransformation in aerobic marine sediments.

Marine Impact of Britannia Mine Sediment: Integrated Sediment Quality Assessment. M.E. Hagen and A.G. Colodey. Environment Canada, Environmental Protection Branch, North Vancouver, BC.

The Britannia Mine is 50 km north of Vancouver, British Columbia, on the east shore of Howe Sound at Britannia Beach. It deposited about 40 million tonnes of tailings onto the foreshore and into the marine environment during its 80 years of operation. Its legacy since closing in 1974 is environmental impact from deposited tailings and on-going discharge of up to three tonnes per day of Cu, Cd and Zn in acidic mine waters. In 2001, the Province of British Columbia reached a $30 million settlement with potentially responsible parties to remediate the site and to construct and operate a water treatment plant. Environment Canada is assessing sediment quality in the areas which are most contaminated based on previous studies and where remediation could take place if necessary. A sequence of subtidal and intertidal sampling and seabed reconnaissance was used to generate chemistry, sediment particle size, metals bioavailability, bioaccumulation, toxicity, and benthic community characteristic results. Along with the traditional sediment quality triad approach, data are linked to georeferenced video, stored on a GIS system, and evaluated using ArcView. Results to date show very high sediment metals in an area north of the Britannia Creek outlet, but relatively low bioavailability and lower toxicity and a richer benthic invertebrate community than expected. Environment Canada data and investigations are linked to uplands contaminated site investigations to give a complete assessment of the site.

Methods for Deriving True Cause-and-Effect-Based Sediment Quality Guidelines. U. Borgmann. Environment Canada, National Water Research Institute, Burlington, ON.

Canadian Interim Sediment Quality Guidelines (ISQGs) for the protection of aquatic life are not based on clear cause-and-effect relationships. Because spiked-sediment toxicity data were limited, the ISQGs were derived from observed correlations between individual contaminants and effects in sediments containing various mixtures of contaminants. Although exceedence of an assessment value may indicate an increased likelihood of toxic effects, correlation is not proof of cause and it cannot be assumed that the contaminant present in excess of the assessment value is necessarily responsible for the observed effects.

An alternative approach to deriving SQGs is to [1] identify methods of quantifying the amount of bioavailable contaminant (e.g., amount bioaccumulated), [2] determine the amount of bioavailable contaminant that causes an effect, and [3] determine the relationship between bioavailable and total contaminant concentrations in the sediment. Sediment assessment values derived for Cd, Cu and Ni in Canadian Shield Lakes near smelters using this approach are considerably higher than those derived using the existing approach, but they are much more diagnostic and indicate the true cause of toxic effects.

Reduced Survival of Early Life Stage Fish Exposed to PAH-Contaminated Sediment. M.E. Bowerman[1], A.E. Winchester[1], J.A. Dungavell[1], C.H. Marvin[2] and P.V. Hodson[1]. [1]Queen's University, Kingston, ON; and [2]Environment Canada, National Water Research Institute, Burlington, ON.

Early life stages (ELS) of fish exposed to PAH-contaminated sediment show increased rates of mortality, CYP1A induction, malformations, reduced growth, and subcutaneous edema in the yolk and pericardial sacs. These abnormalities may inhibit the ability of developing fish to efficiently convert yolk material into biomass. Newly hatched rainbow trout (*Oncorhynchus mykiss*) were exposed to Hamilton Harbour sediment, an area contaminated by PAH from steel industries, for a period of 14 days. Yolk sacs of exposed fish were dissected from the body and wet and dry weight measurements were taken for each. Yolk sac conversion efficiency was calculated using the equation: Efficiency (E) = body weight (14 days) - body weight (hatch) / yolk weight $_{(14\ \mathrm{days})}$ - yolk weight (hatch). Each fish was measured for length and scored for blue sac disease symptoms prior to dissection. Additionally, a sub-sample of exposed fish was preserved for histology and immunohistochemical (IHC) localization of CYP1A protein, an indicator of PAH exposure. IHC analysis involves staining histological sections with monoclonal antibody (MAb) 1-12-3 (donated by J. Stegeman, Woods Hole Oceanographic Institute) in an indirect peroxidase labelling method. The results of these analyses provide an estimate of the relative risk of toxicity to fish of PAH contaminated sediments versus reference sediments.

Development of a New Toxicological Identification and Evaluation Technique using TENAX GR. R. de Vos [1,2]. [1]TNO, Environment, Energy and Process innovation, Department of Ecological Risk Studies, Den Helder, The Netherlands; and [2]Van Hall Institute, Environmental Sciences, Leeuwarden, The Netherlands.

We have investigated the use of TENAX GR, a porous polymer of 2,6-diphenyl-p-phenylene oxide with 30% active carbon to passively absorb bioavailable organic contaminants. The organics absorbed to the passive absorbent TENAX GR are representing the bioavailable organic fraction (tested in TIE phase 1; characterization). This fraction can directly be analysed to indicate the bioavailable toxic pollutant(s) (TIE phase 2; identification). In these experiments, we have used well known polluted sediment from the port of Rotterdam, which is known for its high content of bioavailable organic pollutants (oil fractions C10 to C40). This sediment was manipulated with the standard TIE manipulations and TENAX GR in a best found 1:1 dry weight ratio. The sediment was tested with two bioassays according to a slightly adapted standard protocol, these bioassays were; *Corophium volutator* (marine amphipod) bioassay, and *Vibrio fischeri* bioassay both whole sediment and pore water.

C. volutator mortality in, with TENAX GR manipulated sediment was significantly reduced compared to the pure sediment (25% to 88% mortality, respectively). Effect concentrations measured with *V. fischeri* in a MICROTOX® whole sediment and pore water test were also significantly reduced, compared to pure sediment (whole sediment: EC50: 0.18% pure sediment, EC50: 2.66% TENAX GR manipulated sediment, pore water EC50: 32.5% pure, EC50:>50% TENAX GR manipulated). Chemical analysis of pollutants absorbed by TENAX GR showed that the C-10 to C-23 oil fraction was bioavailable and therefore responsible for the observed toxicity. TENAX GR is a good absorbent for bioavailable toxic pollutants; it reduced toxicity when sediment was manipulated with TENAX GR. chemical analysis showed the bioavailable toxic pollutants.

HepG2 Human Hepatocellular Carcinoma Cell Line Bioassay for Mixtures of Metals and Organic Contaminants in Surficial Sediment Samples from an Area of Concern (AOC) in Lake Ontario and a non-AOC in Lake Erie. E. Diringer, K. Gibbs, J. Giolando, J. Wierchowski and P.F. Dehn. Department of Biology, Canisius College. Buffalo, NY.

The HepG2 cell line has been proposed as a bioassay for aquatic toxicity testing. The purpose of this study was to compare biomarkers in HepG2 cells exposed to real-world mixtures of metals and organochlorines extracted from surficial sediments from an AOC in Lake Ontario and a non-AOC in Lake Erie. Organic and metal extracts were prepared using appropriate EPA methods (3450C, 3620C, and 200.7). Biomarkers of survivorship (Live/Dead) and detoxification capabilities (EROD, PROD, glutathione (GSH), and metallothionein (MT)) were quantified in HepG2 cells after 96 hour exposures. Results were expressed as % of the appropriate controls.

Of the 3 organic fractions tested from each lake, only fractions 2 and 3 from Lake Ontario significantly decreased cell survivorship (45.1±32.6% and 74.7±47.2). EROD activity significantly increased in cells exposed to fractions 2 and 3 from Lake Erie (152.7±18.7% and 141.7±11.6, respectively), while PROD activity was significantly inhibited in all 3 Lake Erie fractions (73.2±22.5%, 70.3±9.6%, and 63.6±12.5%, respectively). Organic extracts did not significantly affect GSH activity. Metal extracts had no appreciable effect on survivorship, while MT levels were significantly higher in cells exposed to Lake Ontario extracts (248.3±118.9%) and GSH activity was significantly higher in cells exposed to Lakes Erie and Ontario extracts (188.4±64.2% and 247.1±79.3%, respectively). Biomarker responses increased as polarity decreased for organic extracts and increased in response to metals. These data suggest that the HepG2 cell line may be a suitable *in vitro* human model for environmental contaminant monitoring.

Comparison of Levels of Heavy Metals in Surficial Sediments and the Game Fish, *Oncorhynchus mykiss*, from an Area Of Concern (AOC) in Lake Ontario and a Non-AOC in Lake Erie via Inductively Coupled Plasma Spectrometry. N. Kibler, J. Snekser, R. Culp, and P.F. Dehn. Department of Biology, Canisius College, Buffalo, NY.

The purpose of this study was to compare levels of Pb, Cd and Hg in surficial sediments and tissues (fat/skin and muscle) from *Oncorhynchus mykiss*, a common game fish, from 18 Mile Creeks in the

Lake Erie basin (non-AOC) and in the Lake Ontario basin (AOC). All fish and sediment samples were extracted and analyzed using EPA method 200.7. Levels of Pb and Hg in surficial sediments from Lake Ontario were 1105±1250 and 8.8±10.3 mg/kg dry weight, respectively, and 450±406 and 11.6 mg/kg dry weight, respectively, from Lake Erie. Levels of Pb, Cd and Hg, in muscle from game fish caught in Lake Ontario were 15.7±25.9, 0.26±0.1, and 6.5±5.5 mg/kg dry weight, respectively, while those from Lake Erie were 0.9, 1.2 ± 0.9, and 4.4±3.3 mg/kg dry weight, respectively. Levels of Pb, Cd and Hg in the fat/skin from Lake Ontario game fish were 9.0±12.8, 0.3±0.1, and 6.0±4.9 mg/kg dry weight, respectively, while those from Lake Erie were 0.7, 0.3, and 6.0±1.1 mg/kg dry weight, respectively. These results generally support our initial hypotheses that: [1] fish and sediments from Lake Ontario would contain higher levels of heavy metals than those from Lake Erie, [2] muscle would contain higher levels of total heavy metals than fat and skin, and [3] sediments would sequester high levels of metals.

GC-Identification of Organochlorine Pesticide Residues in Rainbow Trout, *Oncorhynchus mykiss*, and Surficial Sediments from an Area Of Concern (AOC) in Lake Ontario and a Non-AOC in Lake Erie. R. Frank, G. Kilburn, P. Leone, C. Moise and P.F. Dehn. Department of Biology, Canisius College, Buffalo, NY.

This study was based on Lake Erie LaMP Phase I recommendations, which called for the collection of data of contaminant levels in fish from non-polluted streams as well as AOC's (Areas of Concern), and as such, was designed to examine organochlorine contaminants of historical importance. Sample matrices collected included fat/skin and muscle from rainbow trout and surficial streambed sediment. EPA Methods 3540C and 3620C were used to extract and clean-up, respectively, samples for GC analysis. Levels of organochlorine residues in tissue samples were expressed as mg/kg lipid, while sediment residues were expressed as mg/kg dry weight of sediment extracted. Aldrin, alpha BHC, cis and trans-chlordane, DDD, DDE, DDT, dieldrin, endosulfan I and II, endrin, heptachlor, and methoxychlor were identified in tissue samples.

Fish from Lake Ontario contained a greater diversity of organochlorine pesticides than did those from Lake Erie. Levels of organochlorine residues were higher in fat /skin (0.136 mg/kg to 3.373 mg/kg lipid) than in muscle (0.175 mg/kg to 1.845 mg/kg lipid). Levels of total organochlorines found in fish from Lake Ontario were higher than those of fish from Lake Erie (10.2 vs 3.7 mg/kg lipid, respectively). Sediments contained virtually no detectable organochlorines, with the exception of Lake Ontario where delta BHC was quantified. Our results supported our initial hypotheses that: [1] contaminant levels in the fat/skin would be higher than in the fillets, [2] contaminant levels in fish caught from an AOC would be higher than those from a non-AOC, and [3] surficial sediments would not contain appreciable levels of organochlorine pesticides of historical importance, while tissue would.

Use of Thin Film Solid Phase Extraction to Measure Fugacities of Organic Contaminants in Field Collected Sediments. L. Meloche and F.A.P.C. Gobas. School of Resource and Environmental Management, Simon Fraser University, Burnaby, BC.

Fugacity is a useful tool for measuring chemical partitioning and dynamics in the aquatic environment, and may provide a better prediction of contaminant bioavailability than concentration. Thin Film Solid Phase Extraction provides a relatively rapid and simple means to obtain fugacity measurements of organic chemicals in sediment. The application of this method involves placing sediments in vials coated with a solid phase extracting medium, ethylene vinyl acetate (EVA) in this case. Once equilibrium is reached, the fugacity in the EVA (fe) is equal to that in the sediment and the fugacity in the sediment (fs) can be determined from the EVA-air partition coefficient, which is determined by calibration. We will be presenting results showing the application of the method to determine the fugacity of PCBs in spiked as well as field-collected sediments from False Creek in Vancouver, British Columbia. The results show that equilibrium times are relatively short (less than 48 hours) due to the large surface-to-volume ratio of the films. Relationships between equilibration times and Kow are presented. Method detection limits, field concentration and fugacities are reported for a range of chlorinated halogenated organics. It is concluded that thin films provide a very simple methodology to measure the concentration in the sediments that are likely to be absorbable by aquatic organisms. Uptake experiments in benthic invertebrates are planned to confirm the thin-film measurements of bioavailable chemical fractions in the sediments.

Sediment Bioremediation of Shing Mun River. L. Chan[1], J. Guo[2] and T.P. Murphy[2]. [1]CHEC-OWT JV, Hong Kong; and [2]Environment Canada, National Water Research Institute, Burlington, ON.

The Shing Mun River of Hong Kong had been severely contaminated primarily by the discharge of sewage, resulting in bad odor and algae blooms. The AVS (acid volatile Sulfide) contents in the sediment typically ranged between 1,000 to over 7,000 mg/kg (dry weight). As part of the SMR Environmental Improvement Project, *in-situ* sediment bioremediation is being carried out on 20 hectares of riverbed, using Environment Canada's Limnofix technology.

This treatment method injects calcium nitrate into the sediment to oxidize the sulfide, subsequently reducing the toxicity and promoting the re-establishment of a healthy sediment ecosystem. A barge-mounted injector boom has been used for the pilot and full-scale application. The manoeuvre of the injection barge is controlled by four winches under the guidance of a GPS (global positioning system) navigation system. In monitoring the injection process, concentration profiles of the chemical injected into the sediment were generated through the on-site analysis of core samples collected immediately after the injection process. Analysis of core samples collected 30 days after the chemical injection indicated satisfactory treatment efficiency (AVS reduction rate >99.5%).

Does Bioaccumulation and/or Biomagnification Occur in the Goby and the Zebra Mussels that Live Next to Contaminated Sediments in the St. Clair River? T. Moran[1], J. Houtby[1], T. Kierstead[1], B.A. Zajdlik[2] and S. Munro[3]. [1]Pollutech Eviroquatics Ltd., Point Edward, ON; [2]B. Zajdlik and Associates, Rockwood, ON; and [3]Sarnia Lambton Environmental Association, Sarnia, ON.

Under the Great Lakes Water Quality Agreement, the St. Clair River was designated as an area of concern. A number of impairments are related to contaminated sediments. This project measured the body burdens of selected contaminants in the round goby, *Neogobius melanostomus*, and the zebra mussel *Dreissena polymorpha*. Both of these introduced organisms are common in the St. Clair River and have become critical components in the ecology of the St. Clair River. They may also be critical in the transfer of contaminants from sediments to pelagic fish. The contaminants of concern are those greater than provincial sediment guidelines at some locations and have the potential for bioaccumulation. The list includes metals (Hg, Cd, Cu and Zn), chlorinated organic compounds (total PCBs, hexachlorobenzene, hexachlorobutadiene, octachlorostyrene), and PAHs. Sediment samples and zebra mussels were collected by diver, while the gobies were collected by minnow traps. This study provides up-to-date information on bioaccumulation in two highly abundant taxa that likely play a substantive role in nutrient and possibly contaminant cycling, in the St. Clair River ecosystem. The data set generated may be useful for addressing risks associated with bioaccumulative contaminants of concern.

The Comparative Toxicity of Orimulsion® and Fuel Oil No. 6 to Freshwater and Marine Amphipods. P.M. Jackman[1], K.G. Doe[1] and I. Johnson[2]. [1]Environment Canada, Environmental Science Centre, Moncton, NB; and [2]Golder Associates Inc., Gainesville, FL.

Orimulsion® is a bitumen-based fuel being transported and used worldwide as an alternative fuel for power plant electric generation. Orimulsion® is a mixture of approximately 70 % bitumen, 30% water, and <0.2 %surfactant package. Based on the increased use of Orimulsion® in electric power production, a significant effort has been devoted to research on the chemical and physical characteristics of Orimulsion® as well as its potential ecological effects. New Brunswick Power (NB Power) is in the process of refurbishing the Coleson Cove Plant near Saint John to burn Orimulsion® instead of Fuel Oil No. 6. NB Power has received approval from the Public Utilities Board for this project and has initiated a full provincial Environmental Impact Assessment (EIA). As part of the EIA, NB Power commissioned a comparative ecological risk assessment project to evaluate the potential effects of hypothetical Orimulsion® and Fuel Oil No. 6 spills in the Bay of Fundy. The Environment Canada Toxicology Laboratory in Moncton and Golder Associates have been studying the effects of both Orimulsion® and Fuel Oil No. 6 on the freshwater amphipod, *Hyalella azteca*, and the marine amphipod, *Corophium volutator*. Sediment toxicity tests were conducted using both fuels spiked into uncontaminated sediment. Results of these toxicity tests will be presented, and their implications in case of an accidental spill will be discussed.

Lack of Metals Bioavailability and Toxicity in Subaqueous Tailings, Bluebell Mine, Kootenay Lake. R.F. Baker[1], G.S. Mann[2] and F. Wang[2]. [1]Azimuth Consulting Group, Vancouver, BC; and [2]University of Manitoba, Winnipeg, MB.

The Bluebell Mine, Kootenay Lake, BC operated discontinuously from 1880 to 1972. More than 1.4 million tons of tailings containing high concentrations of As, Cd, Cu, Pb and Zn were discharged to Galena Bay and Kootenay Lake. A sediment quality triad conducted in 2000 identified localized effects (i.e., some toxicity and impaired benthic community) related to spilled concentrate. Otherwise, despite very high metals and metalloid concentrations, minimal impairment of the benthic community and very little toxicity was observed. The pattern of toxicity and benthic community impairment was not explained by either acid-volatile sulphide (AVS) or organic carbon sediment binding phases. Further studies identified iron oxides (Fe-Ox) as the primary metals binding agent and resulted in a strong dose-response relationship with toxicity results. Results suggested that surface complexation with Fe-Ox was responsible for low pore water metals concentrations and reduced bioavailability of metals in Galena Bay and Kootenay Lake sediments. We concluded that bulk sediment concentrations were very poor indicators of effects and that Fe-Ox can be more important binding phases for metals in sediments than TOC and AVS. Subaqueous tailings disposal appears to have been an effective means of mitigating ARD and maximizing long-term stability of Bluebell Mine wastes.

Toxicity Tests on Mercury-Impacted Sediments Adjacent to a Former Chlor-Alkali Plant. M.J. McLeay and W.S. Bosworth. URS Corporation, Vancouver, BC.

Intertidal sediments immediately adjacent to a former mercury-cell chlor-alkali plant in Squamish, British Columbia, were tested in the laboratory to evaluate their potential toxicity to benthic invertebrates. Each sediment sample was the subject of a marine amphipod (*Eohaustorius estuarius*) 10 day survival test, and a marine polychaete worm (*Neanthes arenaceodentata*) 20 day survival-and-growth test.

Plant-site test sediments ranged from 0.561 to 14.8 mg/kg total Hg, well above the Provincial and Federal (Canadian) benchmark values of 0.13 mg/kg (Interim Sediment Quality Guideline = Threshold Effects Level), 0.42 mg/kg (BC Draft Level 1 Criteria), and 0.7 mg/kg (Probable Effects Level) for this metal. Reference sediments contained 0.006 to 0.026 mg/kg total Hg, slightly less than near-site background concentrations. Survival of *E. estuarius* and *N. arenaceodentata* in all sediment samples was high, at 83.0±12.0% and 96.0±8.9%, respectively. One sample produced a statistically significant reduction in amphipod survival. Growth of *Neanthes* in six of the seven Hg-contaminated sediments was unaffected, relative to reference sediments. However, four of the seven Hg-contaminated sediments slightly reduced the surviving amphipods' ability to rebury in clean control sediment. Collectively, the results of toxicity tests at this site are similar to those reported by other authors, and provide evidence that the Federal Interim Sediment Quality Guideline (0.13 mg/kg) and BC Draft Tier 1 Criteria (0.42 mg/kg) for total Hg, may be overly conservative at this site.

Non-Point Source Pollution

Session Chair: P.S. Ross

Identifying Non-Point Source *Escherichia coli* Pollution in Complex Natural Environments.

S. Seurinck[1], W. Verstraete[1] and S.D. Siciliano[2]. [1]Laboratory of Microbial Ecology and Technology (LabMET), Ghent University, Ghent, Belgium; and [2]Department of Soil Science, University of Saskatchewan, Saskatoon, SK.

Despite efforts to minimize fecal input into waterways, fecal contamination continues to be a problem due to an inability to reliably identify non-point sources requiring remedial action. In order to focus public health management programs, identification of the fecal contamination source is crucial. This study evaluates rep-PCR and 16S-23S rRNA intergenic spacer region (ISR) analysis of *Escherichia coli* isolates as a suitable marker for long term ecological monitoring of diverse fecal material. *E. coli* was isolated out of wastewater treatment plant (WWTP)-influent as well as the feces of dogs, horses, gulls and cows. Calculation of the Chao2 richness estimator on the dataset of rep –and ISR patterns showed that for WWTP-influent, horse and gull, 32 isolates was sufficient to characterize the *E. coli* community. However, rep –and ISR analysis gave conflicting results for dog and cow. Analysis of ca. 300 *E. coli* isolates collected, indicated that *E. coli* isolates could reliably differentiate between fecal sources using discriminant analysis. We also evaluated the stability of the genetic fingerprints by comparing rep –and ISR patterns of *E. coli* isolates, before and after 150 days incubation in sterile river water at three different temperatures (4, 12 and 28°C). No change in rep-patterns was observed at 4°C and 28°C. However, major shifts in bands, or loss of bands were observed at 12°C. The stability of the ISR-patterns will also be reported. A pilot study to evaluate the potential for new molecular technologies like real-time PCR to provide same day identification of contamination source in a complex coastal environment is also described. Our results suggest that genetic markers such as ISR or rep-PCR may be appropriate to identify regional sources of *E. coli* contamination in complex watersheds.

Natural Sources of Toxic Substances. G.L. Brown, G.M. Ferguson, R.L. Moore and R.F. Willes. Cantox Environmental Inc., Mississauga, ON.

Understanding the sources of toxic chemicals is one important factor in the assessment of their potential environmental impacts, establishing levels of regulatory and public concerns and the development of appropriate environmental mitigation strategies. There are a large number of natural sources of toxic substances (i.e., sources independent of human activities) that are released into the environment in substantial quantities. Generally these natural releases occur over large areas of the earth's surface. Thus, their environmental concentrations remain small in relation to the focal releases that result in larger concentrations of the same chemicals from anthropogenic sources. However, when considering potential broad-based impacts from these chemicals (e.g., the "greenhouse effect" on global warming), the impacts from large masses of chemicals released from natural sources can, in some cases, be greater than the impacts of these same classes of chemicals from anthropogenic sources. This presentation will provide an overview of the available data on natural sources of chemicals found in water and air. The prime objective of the presentation will be to provide examples of how knowledge of the natural sources of chemicals provides an important perspective on understanding environmental processes, the development of realistic regulatory strategies for the mitigation of potential impacts due to chemicals from anthropogenic sources, and understanding of chemicals in the environment by the scientific community and the general public.

Metal Distribution and Speciation in Stormwater Chamber Systems and Marine Receiving Waters. T. Cohen. Departments of Oceanography and Zoology, University of British Columbia, Vancouver, BC.

Stormwater runoff has been managed along the shores of North Vancouver through the use of subsurface concrete sediment chambers in an attempt to reduce the input of anthropogenic materials to marine receiving waters. These chambers facilitate the settling and accumulation of particle and colloid-bound metal and organic pollutants from stormwater runoff in urban and industrial areas. Metal lability is critical to understanding environmental impacts due to metal pollution, and thus this study sought to examine water and sediment metal speciation and distribution in relation to the presence of stormwater chambers. The field study consisted of measuring levels of Cu, Cd, Pb, Ni, and Zn concentrations at two sites representing different land use areas (industrial and residential) with a more extensive examination of the latter. Metal lability in water was estimated using the diffusive gradient in thin films (DGT). Three techniques were evaluated in the estimation of sediment metal lability (DGT, porewater peepers, and weak HCl acid extractions). In addition, biota metal loads were measured in several aquatic species (barnacles, algae, and mussels) and evaluated for their use as biomonitoring tools. The results of the study will be presented and discussed in relation to the effectiveness of sediment chambers and within the context of broader environmental concerns.

Forensic Analysis of Hydrocarbons in Freshwater Sediment. H. de Pennart and R.A. Crowther. Alpine Environmental Consulting Ltd., Calgary, AB.

Abstract

In July 2000, the rupture of a pipeline resulted in the release of light crude oil into the Pine River in northeastern British Columbia. Chemical analyses showed the presence of polycyclic aromatic hydrocarbons (PAHs) in the sediment. In the absence of baseline data on the sediment quality in the Pine River, an in-depth analysis of the PAHs was used to determine if the system had returned

to pre-spill conditions. PAHs from sediment samples collected in the Pine River and its tributaries were analyzed and the ratios between PAHs known as weathering covariants were computed. In most cases, these ratios did not match the ratios from the spilled oil, rather they matched those of coal samples collected in the Pine River Valley. These results were confirmed by a comparison of the PAH fingerprints obtained from the sediment with those obtained from the spilled oil and coal.

Introduction

On July 31, 2000, a Pembina Pipeline Corporation light crude oil pipeline ruptured, approximately 80 km west of the Town of Chetwynd in northeastern British Columbia, releasing 950 m^3 of product. Following the spill, an estimated 445 m^3 of oil entered the Pine River. Immediately following the spill, an emergency response plan was implemented and booms were deployed along the river to intercept and recover the spilled oil. The initial and primary control point was established approximately 25 km downstream of the break site with four additional interception points further downstream. A mass balance study revealed that following cleanup operations, approximately 78 m^3 of oil remained unaccounted for. An environmental impact assessment (EIA) was implemented in the first few days following the incident to assess the impact of the spill on the Pine River ecosystem.

End points were developed for the sediment component of the EIA, which focused on returning the sediment concentration of hydrocarbons to pre-spill levels. However, as the analytical data was being interpreted, it became clear that apart from the spilled oil, other hydrocarbon sources were present and were contributing to the total sediment contaminant load in the Pine River. In an attempt to document and define possible sources of hydrocarbons and their contribution to the Pine River, the initial sediment assessment program was expanded to include six major tributaries. In addition, the absence of background data for the Pine River and a history of hydrocarbon spills justified the need to conduct an in-depth analysis to identify the types and possible origin of the hydrocarbons found in the Pine River sediments. This analysis was based on polycyclic aromatic hydrocarbons (PAHs) fingerprinting techniques derived from studies conducted as a result of the 1989 Exxon Valdez oil spill in the Gulf of Alaska (Page et al., 1995).

To determine if the PAH concentrations found in the Pine River sediments have reached pre-spill levels, it was necessary to separate the PAHs from the spilled oil from natural and other possible sources. Several natural and/or anthropogenic sources potentially contributed PAHs to the Pine River. The prevalence of coal seams in the Pine River Valley suggested that coal could be a major source of PAH. Emphasis was therefore put on the distinction between PAHs of oil and of coal origin. This paper present the findings from the sediment data collected in the Pine River and its tributaries for the period August 2000 to July 2002.

Materials and Methods

Study design. Impacts were measured by analysis of hydrocarbon concentrations in fine sediments over distance from the spill site and over time. For comparative purposes, sites downstream of the spill site were compared to benchmark hydrocarbon concentrations at a reference site established on the Pine River above the spill zone.

Sampling Stations. All sample sites could be characterized as being erosional sites but were more similar to runs and glides than riffles. Twelve sampling stations were established along the Pine River over a distance of 180 km. Six additional sampling stations were established on the following tributaries to the Pine River: Callazon Creek, Willow Creek, Hasler Creek, Centurion Creek, Sukunka River and Murray River.

Sediment sampling. Ten discrete core samples were collected at, or above the waterline at each sample site using modified Shelby tubes. Core samples were sealed, marked to indicate top and bottom and frozen on dry ice until delivered to the laboratory for analysis. Within the laboratory the 15 cm cores were sectioned into upper, mid and lower sections of 5 cm each. Each section was bisected with particle size analysis done on one half and chemical analysis on the second.

Analytical Chemistry Methods. Samples collected were submitted to a Canadian Association for Environmental Analytical Laboratories (CAEAL) accredited laboratory, for analysis of PAHs, alkylated PAHs, organic content and particle size analysis. PAH analyses were based on U.S Environmental Protection Agency (EPA) method 3540 (for extraction) and EPA method 8270 (for GC/MS analysis).

Interpretive Methods. Previous studies on PAH degradation have shown that, during the weathering process, some compounds degrade at the same rate (Page et al., 1995, Douglas et al., 1996). These compounds, some of which were identified by Hostettler et al., (1999) are called weathering covariants (WC). Two weathering covariants present in the spilled oil were C_2-substituted phenanthrene (C2-Ph) and C_2-substituted dibenzothiophene (C2-DBT). The ratio between C2-Ph and C2-DBT, based on the Hostettler et al., (1999) studies should remain constant throughout the degradation process. Both WC compounds were also found in coal samples collected from the Pine River Valley. PAHs from previous spills or other sources were also expected to contain C2-Ph and C2-DBT.

Weathering covariant ratio calculation. The C2-Ph/C2-DBT ratio was computed for each sediment sample collected during the 2001 and 2002 field program. Only when the C2-Ph or C2- DBT concentrations were ≥ 2 x MDL (Method Detection Limit) was the ratio calculated. The samples were then ranked accordingly to their C2-Ph/C2-DBT ratio. One hundred and twenty eight sediment samples were used for the analysis. C2-Ph/C2-DBT ratio was also computed for samples of oil retrieved from Pembina's pipeline at the time of the break and on Pine Valley (Willow Creek) coal from exposed seams.

Logarithmic regression analysis. Since the C2-Ph/C2-DBT ratio for a specific hydrocarbon source is invariant, all sediment samples contaminated by a given source tend to group together and will plot as a separate line in a logarithmic regression analysis (Page et al., 1995). Each regression line generated by this process is specific to a type of hydrocarbon. In a simple example, samples contaminated with hydrocarbon A or B will plot along regression lines A and B, respectively. However, samples contaminated with a mixture of hydrocarbon A and B will plot between the two regression lines.

PAH fingerprinting. Each source of hydrocarbon has a specific PAH fingerprint and weathered crude oil typically contains more alkylated PAHs than the respective parent compound. As the weathering process occurs, the ratio between the different PAHs families varies, as does the ratio between alkylated PAHs and their parent compounds within each PAH family (Page et al., 1995; Douglas et al., 1996). Fingerprints were developed for coal and oil (Fig. 3A) from the Pembina pipeline, based on the relative, or proportional, concentrations of target alkylated PAHs in each.

Graphical representation. Using the alkylated PAH concentration values for weathered oil and coal, a base template radar plot was created that displays the concentration of each target compound plotted on its parameter specific axis. The radar plot is then used to display the distinctive and unique pattern characteristic of each fingerprinted hydrocarbon source or type (Fig. 3B and 3C). Radar plots were then generated for the data collected from the discrete sediment samples.

Figure 1A: BC PAH Concentration in Pine River Sediment - August 2000
(11 Compounds Analyzed)

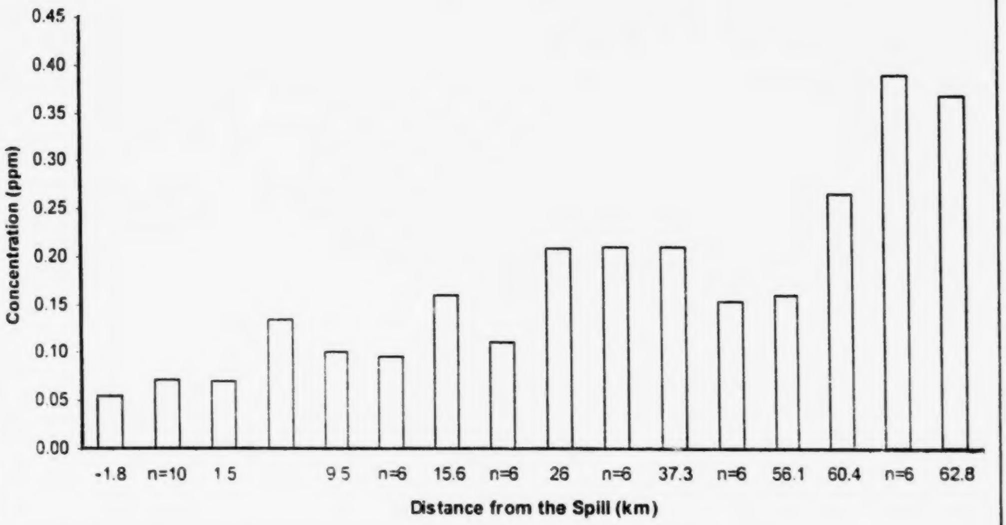
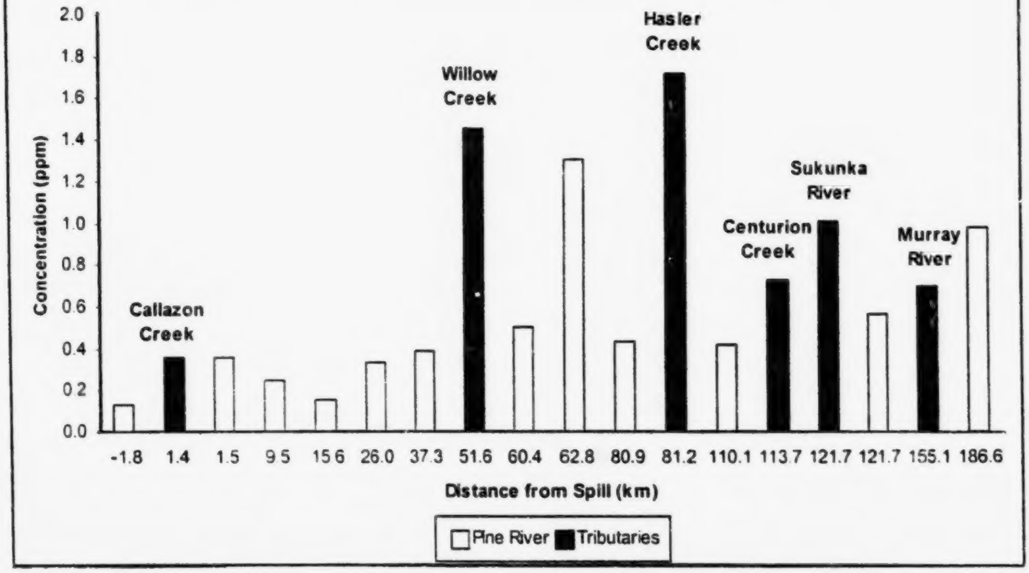


Figure 1B: PAH Concentrations in Pine River and Tributaries Sediment - July 2001
(25 Compounds Analyzed)



Because the PAH concentrations found in sediments were substantially less than is found in the parent source materials, to enable the pattern to be displayed, a site specific factor was applied to the concentration data to expand the scale. This data manipulation allowed the site specific sediment concentration data for the target alkylated PAHs to be overlaid on the radar plotted fingerprints of weathered oil and coal so that similarities and differences in the patterns could be visually discerned. The abbreviations used and shown for the radar plots are: Bph: biphenyl; DBT: dibenzothiophene; Ph: phenanthrene; Na: Naphthalene; Fl: fluorene; and, m: methyl (Table 1).

Results

As a result of the 2000 oil spill, it was expected that hydrocarbons (PAHs) would be found in the Pine River sediment and that concentrations would follow a normal distribution pattern centered on the main control point that was located approximately 25 km downstream of the spill. Results from the August 2000 survey showed that although PAHs were detected in sediments in the upper reaches of the Pine River (between 15.6 and 37.3 km downstream of the spill site) with a peak in concentration at 25 km, the majority of the hydrocarbons in the sediments were found further downstream, between 60 km and 180 km from the spill site (Fig. 1A). This prompted the sampling of selected tributaries to the Pine River to determine if these streams contributed hydrocarbons to the Pine River sediments. As shown on Figure 1B, all the tributaries sampled had substantial

Table 1

Compound			Abbreviations used	# Rings	Concentration in spilled oil (ppm)	Concentration in Pine Valley Coal (ppm)
biphenyl		o	Bph	2	100	0.86
C1-biphenyl		o	m-Bph	2	140	0.31
C2-biphenyl		o	C2-Bph	2	170	0.94
naphthalene	*	o	Na	2	360	1.3
C1-naphthalene		o	m-Na	2	1600	1.8
C2-naphthalene		o	C2-Na	2	3300	0.69
C3-naphthalene		o	C3-Na	2	2300	0.3
C4-naphthalene		o	C4-Na	2	170	0.03
fluorene	*			3	47	0.55
C1-fluorene		o	M-Fl	3	220	0.08
phenanthrene/anthracene	*	o	Ph	3	210	0.79
C1-phenanthrene/anthracene		o	m-Ph	3	530	3
C2-phenanthrene/anthracene		o	C2-Ph	3	1100	1.7
dibenzothiophene		o	DBT	3	180	0.18
C1-dibenzothiophene		o	m-DBT	3	610	0.23
C2-dibenzothiophene		o	C2-DBT	3	1200	0.57
C3-dibenzothiophene		o	C3-DBT	3	810	1.1
pyrene	*			4	9	0.17
benzo(a)anthracene	*			4	5.8	0.18
chrysene	*			4	18	0.35
benzo(b)fluoranthene	*			5	<1	0.1
benzo(k)fluoranthene	*			5	<1	0.03
benzo(a)pyrene	*			5	<1	0.07
dibenzo(a,h)anthracene	*			5	<1	0.06
indeno(1,2,3-c,d)pyrene	*			6	<1	0.04

* Compounds regulated by BC Sediment Guidelines

o Compounds Used for Fingerprinting Analyses

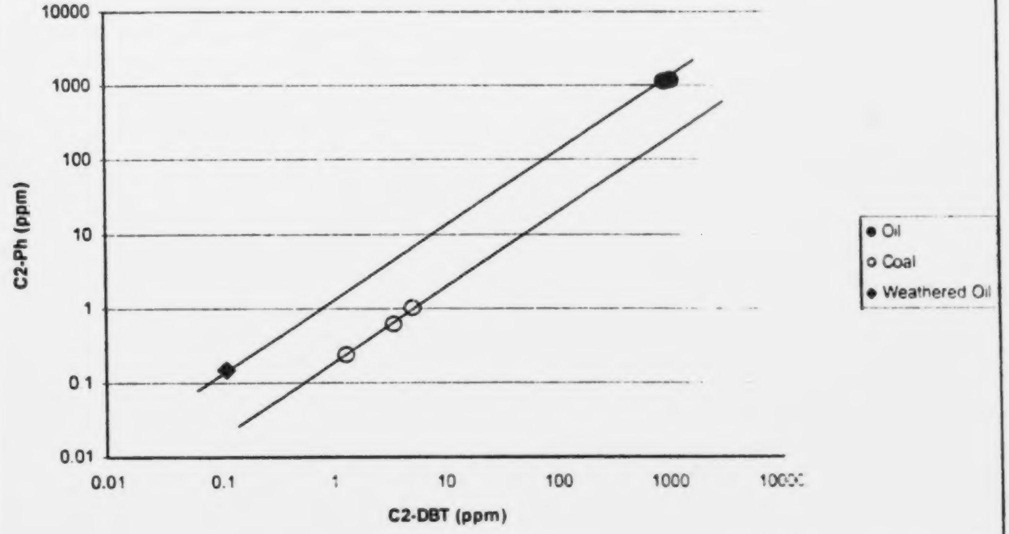


Figure 2A : C2-Phenanthrene/C2-Dibenzothiophene Ratio in Pine River Sediment - 2001

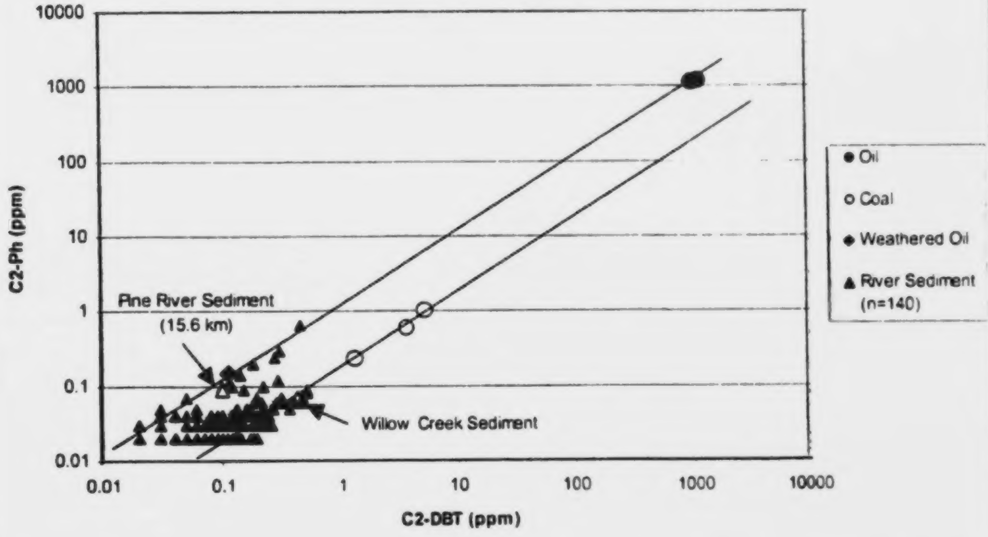


Figure 2B : C2-Phenanthrene/C2-Dibenzothiophene Ratio in Pine River Sediment - 2001

concentrations of PAHs in their sediment in comparison to the concentrations measured in the Pine River sediments (Fig. 1B). Additional PAHs were analyzed in 2001 to account for the compounds that had been found in the oil (Table 1).

The presence of PAHs in the Pine River tributaries was not surprising given the various activities (mining, logging, transportation, etc.) taking place in the Pine Valley corridor. This additional hydrocarbon loading from obviously different sources posed a problem when trying to study the impact of the oil spill on the Pine River sediment and especially to determine if the PAH concentrations have reached pre-spill levels. For this reason, fingerprinting techniques were used to discern PAHs of known oil origin from those of unknown origin. The main source of PAHs in the tributaries was thought to be coal, which is ubiquitous throughout the Pine River and adjacent valleys. In addition, coal is currently mined along Willow Creek and was also mined at Tumbler Ridge, located upstream along the Sukunka River.

In the analysis of both the spilled oil and coal samples collected along Willow Creek, C_2-phenanthrene (C2-Ph) and C_2-dibenzothiophene (C2-DBT), two identified PAH weathering covariants were found (Page et al., 1995). The C2-Ph/C2-DBT ratios were 0.88±0.03 for unweathered and weathered spilled oil and 5.59±0.36 for coal. The C2-Ph and C2-DBT concentrations were plotted on a logarithmic scatterplot (Fig. 2A). Weathered spilled oil plotted on the same regression line as unweathered spilled oil, whereas the data from coal samples plotted on a separate regression line. The C2-Ph/C2-DBT ratios in all discrete sediment samples collected in 2001 in the Pine River and its tributaries ranged from 0.6 to 9.5. The wide range in ratio is indicative of a large diversity in the sources of PAHs present in the sediment. This diversity was clearly visible on the logarithmic scatterplot (Fig. 2B) where sediment samples did not plot along a single line, but rather were distributed between the regression lines from oil and coal. In part, these results reflect the sediment contribution from each tributary to the hydrocarbon load found in the Pine River sediments. This resulted in an increasingly complex mixture of hydrocarbon when moving downstream, as more tributaries (potentially associated to diverse sources of hydrocarbons) contribute sediments to the Pine River.

Table 2

Population #	Mean C2-Ph/C2-DBT ratio	Coefficient of Correlation R^2	Mean Distance from Spill Site km
1	0.72	0.99	24.69
2	1.08	0.99	33.85
3	1.55	1.00	31.70
4	2.10	1.00	56.21
5	2.50	0.99	70.87
6	3.00	1.00	75.18
7	3.63	0.96	74.69
8	4.81	0.97	91.23
9	5.58	1.00	79.51
10	6.94	0.95	107.01
Coal	5.59	0.99	NA
Oil	0.88	0.99	NA

In order to interpret the data collected from the Pine River sediments, several data populations were generated, based on C2-Ph/C2-DBT ratios. Each population was then tested against the closest one to check for statistical differences. The null hypothesis (H_0), that two populations regression lines are the same, was assessed by a t-test (Zar, 1984). Results of this analysis showed that ten statistically distinct populations could be generated (Table 2). Populations, whose regression lines were close to the spilled oil regression line were found to be composed of samples from sediment collected in the upper reaches of the Pine River. Conversely, the further a population regression line was from that of the spilled oil, the higher the probability that the data was derived from sediment samples collected from the tributaries or from downstream locations along the Pine River. This confirms that any residual spilled oil left in the river sediments in 2001, was confined to the upper reaches of the Pine River (within 40 km from the spill site). The C2-Ph/C2-DBT ratio analysis also confirmed that the Pine River has and is receiving substantial PAH loads from most of its tributaries, which are likely related to coal.

To confirm the relationship of the PAHs found in the Pine River sediment to either oil or coal, a fingerprinting technique based on a graphical representation of a specific hydrocarbon type was developed (Fig. 3). Although the spilled oil and Pine Valley coal shared the same PAH compounds, their distribution and contribution to the fingerprint varied greatly between the two hydrocarbons (Fig. 3A). This difference was clearly visible on the corresponding radar plots (Fig. 3B and 3C), which were used as templates to assess the origin of the PAHs found in the sediment. Figures 3D show the radar plot for sediment collected in the Pine River, approximately 15 km downstream of the spill site. The match between the pattern produced by the sediment PAHs and the spilled oil at this site indicates that some residual oil remain in the Pine River sediments at this location. This particular sample is shown on Fig. 2B where its close relationship to the spilled oil is confirmed. The pattern obtained from sediment collected in Willow Creek (Fig. 3E), showed only a partial match with that of Pine Valley coal (Fig. 3E). The Willow Creek sample and its relationship to Pine Valley coal is shown on Fig. 2B). The difference in the patterns shown on Figure 3E is likely related to the existence of several coal seams, which would likely have different PAH compositions (Schultz et al., 1972).

Discussion

The data presented in this paper shows that, in an event such as an oil spill, the characterization of the spilled oil is of great importance to assist in determining impact on the environment. Only eleven PAHs compounds have criteria in the Freshwater Sediment Criteria for the Protection of Aquatic Life of the British Columbia Water Quality Guidelines (BC WQG, 1998). Since most of these regulated PAHs were not present in the spilled oil, a study limited to these compounds may have lead to erroneous conclusions regarding the impact to Pine River sediment. The absence of background data on the Pine River sediment was also a problem because of the ubiquitous presence of PAHs in the Pine River environment. Fortunately, the fingerprinting techniques developed for this project and described in this paper proved to be a useful tool to discern between PAHs from various sources and origin. This distinction was of great importance and assistance in assessing the effects of the spill on the Pine River ecosystem since the bioavailability of PAHs has been shown to be dependent upon their origin (Chapman et al. 1996). Most of the PAHs detected in the Pine River sediments were found to be of coal origin and were therefore not bioavailable. This was confirmed by results from other components of the Pine River EIA, showing that the effects of the oil spill on benthic invertebrates and periphyton were restricted to the upper reaches of the Pine River (Alpine 2001a, 2001b). These studies and the results from the work presented herein prompted a refocusing of the Pine River sediment sampling program for 2002 to a 50 km stretch of river, immediately downstream of the spill site. The data collected to date showed that the concentration of PAHs from

Figure 3A : PAH fingerprints - Pine Valley Coal vs. Pembina Weathered BC Light Crude

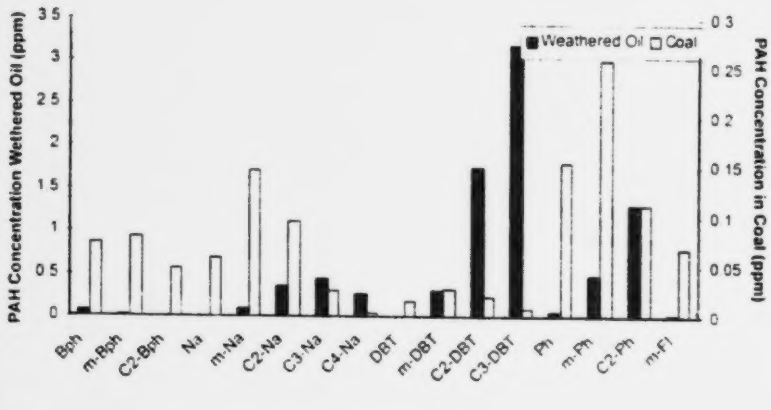


Figure 3B: PAH fingerprints - Pembina Weathered BC Light Crude

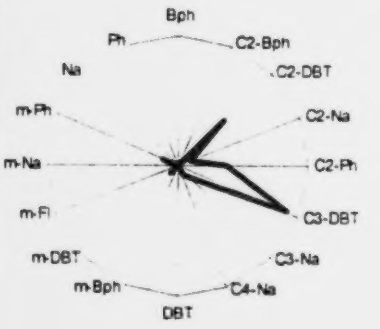


Figure 3C: PAH fingerprints - Pine Valley Coal

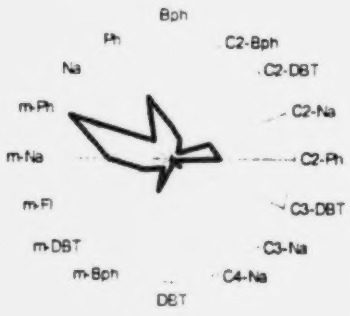


Figure 3D: Fingerprint of Alkylated PAHs Collected in the Pine River Sediment - 2001

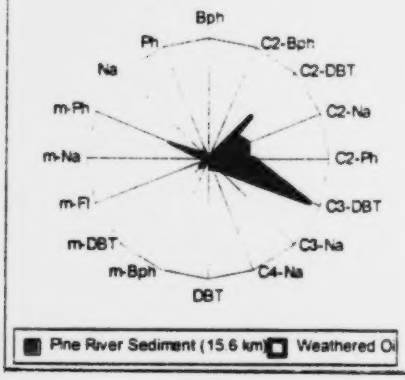
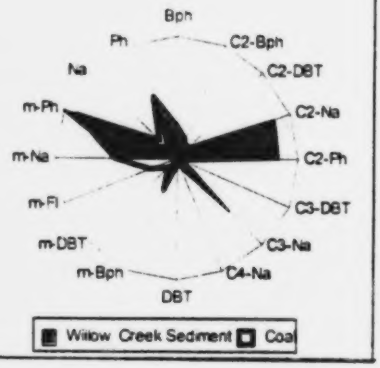


Figure 3E: Fingerprint of Alkylated PAHs Collected in Willow Creek Sediment - 2001



the spilled oil found in the Pine River sediment has decreased dramatically since August 2000. In fact most of the sediment collected in 2002 in the upper reaches of the Pine River had PAHs concentrations so low that it was not possible to conduct a C2-Ph/C2-DBT ratio analysis.

These findings confirm that high-energy streams like the Pine River also have a high self-cleaning ability. The removal of introduced hydrocarbon compounds in such systems is accelerated by continuous re-suspension, aeration and transport of sediments. These mechanisms, in turn, favor the dispersion and biodegradation of hydrocarbons. The other particularity of flowing systems, which results from their unidirectional nature, is the increased complexity of the sediment composition with downstream travel. In the Pine River, this resulted in the increasingly complex PAH mixture found in the sediment as samples were collected at increasing distance from the spill site. This distinctiveness required the adoption of fingerprinting techniques that were originally developed to identify hydrocarbon contaminant sources in marine sediments following oil spills.

Acknowledgements

The authors wish to thank Pembina Pipeline Corporation and Fred Kuipers for the use of their data. The technical support from Enviro-Test Laboratories in Edmonton was also greatly appreciated.

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Diffuse, Semi-Volatile Contaminants and Climate Variability – What Happens When Pathways Change? R.W. Macdonald. Department of Fisheries and Oceans, Institute of Ocean Sciences, Sidney, BC.

The rapid arrival in the Arctic of semivolatile contaminants (PCBs, DDT, toxaphene, Hg) from temperate and tropical countries was initially a great surprise. Much research over the past decade has revealed the pathways in air and water that deliver contaminants to the remotest locations on the planet within days (air) or within years to decades (water). These pathways, however, are inherently diffusive and accompanied by large dilution factors from which one might infer that risk to ecosystems should decrease as distance from the source increases. However, this proves not to be the case because the environment has a number of concentrating processes which ultimately decide whether or not a "diffusive" contaminant provides a risk. An obvious, but almost ignored, implication is that the distribution of contaminants in environmental media may be very much impacted by global change. Here, I will outline a few examples where recent climate change has altered contaminant pathways in the Arctic, and then discuss the response of environmental reservoirs to transient inputs of semi-volatile contaminants, initially providing sinks but then becoming sources when emissions are controlled.

Impacts of Smelt Invasion on Food Web Structure, and Mercury and Persistent Organochlorine Concentrations in Top Predators. K.A. Kidd[1], T. Johnston[2], D.M. Whittle[2], D.C.G. Muir[3] and M.S. Evans[4]. [1]Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, MB; [2]Department of Fisheries and Oceans, Bayfield Institute, Burlington, ON; [3]Environment Canada, National Water Research Institute, Burlington, ON; and [4] Environment Canada, National Water Research Institute, Saskatoon, SK.

In the fall of 1999 and summer of 2000, lake trout, forage fishes, zooplankton and benthic invertebrates were collected from two lakes with smelt (Eva and Sandybeach) and two lakes without smelt (Paguchi and Thunder) in northwestern Ontario. Fish muscle and pooled invertebrates were analysed for C and N isotopes to examine food web relationships, and for Hg and several persistent organic pollutants (POPs). Food web length was not significantly different across the four lakes; mean δ^{15}N of the lake trout ranged from 10.3 to 11.9 per mil, and from 2.0 to 3.3 per mil in clams from each of the sites.

Hg concentrations in lake trout were not significantly different across lakes ($p=0.72$). In contrast, concentrations of DDT, chlordane, lindane, and chlorobenzene (but not PCBs) were significantly higher in lake trout from smelt lakes when compared to the non-smelt lakes. These differences could not be attributed to differences in food chain length or size of the fish, but were related to the higher lipid in trout from lakes with smelt (13 and 14%) versus non-smelt lakes (7 and 8 %). These results indicate that smelt invasion impacts the bioenergetics of food webs and augments the accumulation of POPs in top predators.

Transport and Fate of Contaminants in the Strait of Georgia, British Columbia. S.C. Johannessen, R.W. Macdonald, D. Cullon and P.S. Ross. Department of Fisheries and Oceans, Institute of Ocean Sciences, Sidney, BC.

Persistent organic pollutants and metals have been measured in the sediments and organisms of the Strait of Georgia, and hundreds of new chemicals, including pesticides and pharmaceuticals, enter the strait every year. Despite the ecological and economic importance of the strait, we do not have a comprehensive understanding of the behaviour of contaminants in this system. Many contaminants attach to particles and organic matter, so box model budgets of those components can suggest the most likely pathways and fate of the contaminants. Here, we present a description of particle and organic carbon pathways through the strait, based in part on data from sediment cores and traps, and discuss the implications for contaminant transport.

The Fraser River represents the main source of particles to the Strait of Georgia. Particle flux decreases with distance from the river mouth and is lowest in the northern strait. Dissolved and particulate organic carbon derive principally from in situ primary production and from the Fraser River. Most of the particles, including particulate organic carbon, are buried in the sediments of the strait, while dissolved organic carbon is likely oxidized or transported out to the Pacific Ocean. Bottom currents redistribute the settled particles. Published contaminant measurements suggest that contaminants follow similar pathways. The major contaminant sources are expected to be: rivers, the local atmosphere, municipal and industrial effluents, and exchange with Puget Sound and the Pacific Ocean. Characterizing the sources and pathways of these contaminants represents an important foundation for mitigation strategies and for reducing risks to humans and wildlife.

Marine Mammals as Sentinels of Contamination in the NE Pacific Ocean by Persistent Organic Pollutants. P.S. Ross[1], B. Hickie[2], M. Yunker[1], R.W. Macdonald[1] and S. Jeffries[3]. [1]Department of Fisheries and Oceans, Institute of Ocean Sciences, Sidney, BC; [2]Trent University, Peterborough, ON; and [3]Washington Department of Fish and Wildlife, Tacoma, WA.

Marine mammals often occupy high trophic levels in the marine environment, and can therefore be exposed to high levels of many Persistent Organic Pollutants (POPs). We recently demonstrated that NE Pacific killer whales are among the most PCB-contaminated marine mammals in the world. While the source of these contaminants remains to be elucidated, our current research suggests that a combination of local and global sources are contributing to the contamination of killer whales.

A spatial assessment of POPs in non-migratory harbour seals in BC and Washington State revealed Puget Sound to be a PCB "hotspot". PCB profiles indicate that seals inhabiting Puget Sound are exposed to a "heavier" mixture of congeners (i.e., more chlorinated) than seals inhabiting the Strait of Georgia or Queen Charlotte Strait in adjacent Canadian waters, likely reflecting proximity to sources. However, we have also obtained evidence that BC marine mammals are exposed to a "global" background POPs signal. Chinook salmon, the preferred prey of resident killer whales (estimated at 65% of annual consumption), obtained approximately 98% of their POPs burden from the open Pacific Ocean.

In addition, the "lighter" PCB signature found in seals from remote BC sites is consistent with the importance of atmospheric delivery pathways for such congeners. A 20 year wind reanalysis for our region suggests that prevailing wind currents could readily introduce POPs into remote parts of BC from regional (e.g., Puget Sound) and global (e.g., Asian) sources. A temporal trend model suggests that levels of PCBs have dropped by a factor of approximately 2.5 times between 1970 and 1985 in killer whales, with little change since. Our research underscores the need for further information on the sources, pathways and fate of POPs in the BC environment.

Immunomodulation in Fish Exposed to Different Source Points. S.D. St-Jean[1], S.C. Courtenay[1], F.X. Valdez-Domingos[2] and C.A. Ribeiro[2]. [1]Department of Fisheries and Oceans, Gulf Fisheries Centre, Moncton, NB; and [2]Federal University of Parana, Brazil.

Pictou Harbour in Nova Scotia, Canada, receives liquid effluents from a variety of industrial and municipal sources. In order to characterize the influence of these different anthropogenic inputs of Marine Environmental Health, a community-based partnership of the federal government (Environment Canada and Fisheries and Oceans), an ENGO, the Pictou Harbour Environmental Protection Project, industry (pulp and paper) and operator of a municipal wastewater treatment plant was formed to investigate the utility of immunological bioindicators over a two year period.

Beginning in May 2000, 10 females and 10 males mummichog fish (*Fundulus heteroclitus*) were collected from five potentially impacted sites and a reference site monthly until October and the following measures were taken: Phagocytic Activity (PA); Lysosome Retention (LR); the production of oxidants, H_2O_2 and O_2^- and the production of bactericide in the haemolymph. In addition, total proteins in the blood, body length and weight were monitored concomitantly with the immune protocols. Significant differences were observed between sites in several endpoints during monthly sampling. Furthermore, trends in seasonal changes of immunological endpoints differed between sites. Results suggest that there is sufficient variation over sites and time in immunological endpoints to render them a useful biomarker.

Novel In-Tube Solid Phase Micro Extraction of Aquatic Samples for GC Analysis. R.H. Wohleb and M. Okiro. VICI Gig Harbor Group, Inc., Gig Harbor, WA.

In-tube Solid Phase Micro Extraction (SPME) is a relatively new extraction technique utilizing open tubular capillary GC columns as the extraction medium. Organic compounds are extracted into the liquid phase as aqueous samples are passed through the column. Extracted components may be thermally desorbed directly into a GC injection port or back extracted into a suitable solvent. A new type of in-tube SPME will be presented in which the extraction "phase" is an octadecyl derivatized porous layer similar in selectivity to standard SPE phases. Idealized operating parameters will be presented with comparison to standard in-tube SPME. The applicability of the sampling technique to the capillary GC analysis of pesticide contaminated water will be presented.

Combined Chemical and Toxicological Analysis upon Time-Integrated Passive Sampling. S.K. Bopp and K. Schirmer. Junior Research Group of Molecular Animal Cell Toxicology, UFZ Center for Environmental Research Leipzig-Halle, Leipzig, Germany.

A variety of passive samplers, using liquid or solid receiving sorbents, is currently applied to the monitoring of aquatic pollutants. Most devices are used for chemical analysis only, focusing on known or presumed contaminants. In contrast, biological indicators, such as cultured cells, can indicate the presence of potentially toxic substances in an environmental sample as a whole. Thus, it is our goal to develop a passive sampling device that can be used for combined chemical and biological analysis using a solid receiving sorbent as the common base. Chemical analysis shall be done by thermodesorption or simple solvent extraction. Biological analysis will be based on the direct availability of sorbed contaminants to indicator cells capable of adhering to the solid sorbent. Biosilon (polystyrene), Tenax-TA, Tenax-GR and Chromosorb-106 showed to be suitable for cell adherence whereas Amberlite IRA-743 did not allow cells to attach. For the materials enabling cell adherence, various concentrations of sorbed contaminants elicited dose-response relationships in the adhering cells, thereby demonstrating the bioavailability of the sorbed contaminants. Further important parameters to be considered are the ease of handling and the resistance of the sorbents to chemical extraction. For sampling contaminated water, the sorbent of choice will be inserted into a ceramic dosimeter, an established passive sampling device relying on a ceramic tube as a diffusion barrier.

Histopathological Evaluation of Mummichog (*Fundulus heteroclitus*) (Teleostei, Cyprinodontidae) Collected at Estuarine Sites Receiving Pulp and Paper, Heated and Municipal Waste Water Effluents in Pictou Harbour, Nova Scotia. S.D. St-Jean[1], F.X. Valdez-Domingos[2], S.C. Courtenay[1] and C.A. Ribeiro[2]. [1]Department of Fisheries and Oceans, Gulf Fisheries Centre, Moncton, NB; and [2]Federal University of Parana, Brazil.

Mummichogs (*Fundulus heteroclitus*) were sampled from three different types of effluents: pulp and paper, heated and secondary treated municipal wastewater and from a reference site in the summer of 2000. They were evaluated for histopathological damage of the gills and liver using light and electron scanning microscopy. Fifteen individuals were collected from each site during June to September 2000. The gills and liver were excised to evaluate the waterborne and trophic exposure to the different type of effluents respectively.

Necrosis in the liver was noted at all sites, with the control showing less and the secondary treated sewage effluent showing the most. Coagulative necrosis, shown to be caused by anthropogenic exposure, was noted in the pulp and paper and the secondary treated sewage effluents. In addition, acidiophilic areas were noted in the livers of fish originating from the waste waster effluent. Gills from all sites showed the presence of parasites and gill filament fusion was observed in the heated effluent, presumably an effect of. This study demonstrate the usefulness of histopathological indices in assessing marine environmental quality.

From Source to Tap: The Multi-Barrier Approach to Safe Drinking Water. P. Jiapizian and P.-Y. Caux. Environment Canada, Environmental Quality Branch, Ottawa, ON.

Recent outbreaks of waterborne disease in Walkerton, Ontario, and North Battleford, Saskatchewan, have heightened Canadian awareness of the fact that threats to water quality and quantity can have a profound impact on their health, the environment, and the economy. This poster outlines the elements of a multi-barrier approach which would help ensure Canadian drinking water supplies are kept clean, safe and reliable for generations to come. The multi-barrier approach recognizes the inter-relationship of health and environmental issues, and encourages the integration of efforts to improve public health with those that also protect the natural environment. This poster aims at communicating the concept of a multi-barrier approach to drinking water protection. It builds on the experiences of Canadian and international jurisdictions and serves as a template for the strategic alignment of *Canadian Water Quality Guidelines*, best management practices, research and monitoring with an integrated source to tap approach to drinking water protection. A comprehensive technical supporting document is being prepared and will provide detailed guidance to assist Canadian authorities in implementing the approach. This approach is not meant to be prescriptive but rather should be adapted to reflect the specific needs of a community with regards to providing safe drinking water. It is hoped that this initiative will provide the basis for the on-going integration of health and environmental issues related to drinking water quality and should set the stage for increased collaboration and information-sharing among jurisdictions.

Toxicity Identification Evaluation Case Study of a Groundwater Discharge Facility Located in Orange County, California, US. A. Farmer, C. Stransky and J. Rudolph. AMEC Earth and Environmental, San Diego, CA.

Phase I and Phase II Toxicity Identification Evaluation (TIE) procedures were performed on an historically toxic groundwater sample in the summer and fall of 2001, respectively. The groundwater was routinely pumped from an underground facility and discharged to a freshwater environment. A sample salinity of approximately 14‰, however, excluded the use of freshwater species for toxicity testing due to their lack of physiological tolerance. Therefore, the groundwater was brought to a salinity of 30‰ and tested using a marine shrimp, *Americamysis bahia* (formerly *Mysidopsis bahia*). Results from Phase I testing revealed that none of the classes of contaminants investigated (e.g., filterable materials, nonpolar organics, volatile compounds, oxidants, trace metals) were responsible for the toxicity observed in the sample. Upon further investigation of the literature and chemical evaluation of the sample, an imbalance in the ratio of major seawater ions was suspected of causing toxicity. Subsequent Phase II TIE testing confirmed that ion imbalance, particularly a deficiency in potassium and excess in bicarbonate, were responsible for toxicity in the fall 2001 sample.

Pollution Prevention in Kamloops: Linking Research and Education with Action. M.D. Gillis[1] and W. Archambault[2]. [1]City of Kamloops Engineering, Public Works and Development Branch, Kamloops, BC; and [2]British Columbia Conservation Foundation, Kamloops, BC.

Non Point Source Pollution (NPSP) is difficult to measure and identify. It comes from many sources, and though often in very small quantities, its impacts can add up to be wide ranging and long term. Environmental, economic and cultural benefits of implementing a pollution prevention program far outweigh the costs of NPSP (i.e. degraded water, air and soil quality for flora/fauna, degraded drinking water quality, hindrance to water-based recreation and tourism due to health and safety considerations, and reduction of aesthetic appeal). Since our day-to-day activities contribute to NPSP, education can be a powerful pollution prevention tool.

The City of Kamloops developed an NPSP Education Program as a pilot program in 1997 with an aim to reduce urban pollution by educating the public and thereby potentially improving the quality of water in the Thompson Rivers. Since then, the program has been expanding to address air quality, climate change, integrated pest management, and more. The NPSP Education Program goals center on utilizing science and research to excite and catalyze both the community, and the City as a corporation, to take action to reduce and prevent pollution. Kamloops is having fun and experiencing success using hands-on workshops and interactive watershed models, designing websites, promoting Awesome Alternatives, facilitating tours, creating Green Smartees and Car Time Calculators, and hosting its first ever on-line newsletter - the Pollution Solution. To record and monitor its efforts, the City is currently developing an educational database that it hopes will become a resource for other communities who want to encourage pollution prevention.

One ingredient for the success of the NPSP Education Program is the linkages to other programs such as WaterSmart, TravelSmart, the Environmental Indicators Program, Partners for Climate Protection Program, Habitat Conservation and Stewardship Program, Interagency Team on Environmental Protection, Environmental Performance Advisory Committee, and the Environmental Management System (currently being developed to enhance communications, improve efficiency, increase confidence and pride, encourage public participation, foster continual improvement, encourage action within the community, and attempt to monitor and measure success). Other ingredients include the energy, creativity and compassion of the undergraduate science students who have coordinated this program, and the support from the City of Kamloops, British Columbia Conservation Foundation, Ministry of Water, Land and Air Protection, University College of the Cariboo, Fisheries and Oceans Canada, and other partners and volunteers.

Georgia Basin Mussel Watch — A Proposal for Integrated Biomonitoring. M.H. Salazar and S.M. Salazar. Applied Biomonitoring, Kirkland, WA.

Several Georgia Basin monitoring programs in the US and Canada are designed to quantify the status and trends in ambient conditions using long-term monitoring approaches. A Georgia Basin Mussel Watch program is proposed to better integrate the biomonitoring elements of these programs. "Mussel Watch" is a monitoring approach that typically includes measuring tissue chemistry in resident or transplanted bivalves at regular intervals to establish the status and trends in environmental quality. The proposed approach would include measuring other endpoints such as biomarkers and growth to add an effects component to the monitoring. The addition of a Georgia Basin Mussel Watch Program would provide a method to focus these programs on a more common goal, minimize the costs of data collection and maximize the consistency of the protocols. Other benefits to establishing a basin-wide Mussel Watch program include integration of existing programs with more cost- and services-sharing, consistency with the risk assessment methodology, more emphasis on using other tools in the environmental monitoring toolbox, and addition of previously under-utilized monitoring species. The purpose of this poster is to focus on rationale and methods for establishing a Georgia Basin Mussel Watch Monitoring Program, and to make specific recommendations for implementation. Examples will be provided where similar approaches have been used in the US and the UK using both indigenous and caged bivalves over similar temporal and spatial scales.

Seasonal Influences on Toluene Concentrations in the St. Clair River. S. Munro[1], E.J. Kuley[2], W.R. Brooks[2] and T. Moran[3]. [1]Sarnia-Lambton Environmental Association, Sarnia, ON; [2]Canadian ORTECH Environmental Sarnia, ON; and [3]Pollutech EnviroQuatics, Point Edward, ON.

Abstract

The Sarnia-Lambton Environmental Association has maintained a program of fully automated hourly sampling of the St. Clair River downstream of industrial effluent discharges for 16 years. The monitoring system consists of a purge-and-trap sample concentrator coupled to an automatic stream sampler. Analysis is by on-site gas chromatograph with parallel flame ionization and electron capture detectors. Detector output is fed to a computing integrator, with results reported hourly by telemetry to a central database.

Toluene, typically within the range of 0.1 to 0.5 µg/L, appears most frequently of the 20 analytes, exhibiting consistent seasonal, weekly and diurnal patterns over several years. The pattern implies pleasure boat activity on the upper reaches of the St. Clair River as the source of the toluene, rather than industrial discharges from the petrochemical industry. Results of additional sampling across a transect of the river perpendicular to the permanent sampling location and a review of industrial discharges strengthen the association with pleasure boating. Based on river flow and ambient analyses, annual toluene loading to the river from pleasure boating appears to be an order of magnitude higher than that attributable to industry.

Introduction

The St. Clair River is a 64 kilometer long connecting channel flowing southward from Lake Huron to Lake St. Clair. Average volumetric flow is about 5200 m^3/sec, with current velocities ranging between 0.6 and 1.8 m/sec. Flow time from Lake Huron to Lake St. Clair is about 21.1 hours (St. Clair River Remedial Action Plan Team, 1991). The river forms the international border between Michigan, USA and Ontario, Canada.

There are 11 municipal sewage treatment plants (5 US; 6 Cdn) and 33 industrial discharges (6 US; 27 Cdn) to the river. Industrial discharges are predominately from petroleum refining, petrochemical and coal-fired thermal electric generation facilities. Refining of local crude oil began in the 1860s. Major expansions of refineries and petrochemical facilities occurred in the 1940s, 1950s and 1970s. Non-point sources of contaminants include urban and rural runoff, combined sewer overflows, waste disposal site runoff and leachate, contaminated sediment, commercial shipping, and pleasure boating. River flow characteristics confine point source discharges to the five per cent of the river's volume along each of the shorelines (St. Clair River Remedial Action Plan Team, 1991), with 90 per cent in the central shipping channel.

A database of hourly monitoring records for 20 volatile organic compounds (VOCs) typical of petrochemical discharges has been maintained since 1986. The frequency of detection of toluene in the range of 0.01 to 0.5 µg/L is much higher than expected, based on known industrial inputs (Environment Canada). A review of the database led to speculation that pleasure boating on the St. Clair River and in lower Lake Huron contributed to the frequency with which toluene was detected. Further, toluene from boats should be fully mixed throughout the river.

Materials and Methods

The remote, unattended automated VOC monitoring system is located 18 kilometres south of Lake Huron, downstream of all known industrial sources of toluene. The intake is 40 meters offshore, at a depth of three meters. Experience has demonstrated that the sampling point is representative of water quality in the five per cent of river flow carrying effluents from Canadian shore-based facilities (St. Clair River Remedial Action Plan Team, 1991;Kuley and Brooks, 2001).

The remote system continuously circulates 100 L/min from the intake. A Tekmar APS automatic stream sampler initiates a five ml aliquot once per hour. An internal standard, bromodichloromethane, is injected into each aliquot. VOCs are purged with helium onto a carbopackB/carbosieveC trap (Tekmar Model LSC-2000). EPA method 602 (1982) for VOCs, modified for unattended remote application. The trap is thermally desorbed to a 30 metre DB-624 megabore capillary column, with detection by flame ionization and electron capture running in parallel. The method detection limit (US Federal Register, 1984; Kuley and Brooks, 2001) is 0.08 µg/L.

To test the homogeneity of toluene mixing through the river, an east-west transect across the river at the fixed sampling location was established. Grab sampling following standard procedures using a Van Dorn sampler was completed at three depths at each of five locations across the transect. Sampling was conducted at approximately 23:00 hours, corresponding to the expected diurnal peak, on two occasions. A weekend evening in April, prior to the boating season, and a weekend evening in early September, in full boating season, were chosen. Samples were analysed by manual injection into the automated system described above, within 12 hours of collecting the samples.

Results and Discussion

Long-term trend monitoring of St. Clair River water quality has identified toluene as the contaminant most frequently observed above detection limits among 20 VOCs analysed hourly. Table 1 summarizes results for the period 1996 to 2001 for which method detection limits have been consistent. All toluene concentrations reported through the period of study were below the Ontario Provincial Water Quality Objective (1999) of 0.8 µg/L and Ontario Drinking Water Standard (2001) of 24 µg/L for aesthetic reasons.

Based on hourly sampling at the fixed sampling location, the frequency of detection of toluene was seasonal, peaking in the warmest months (Fig. 1). Within the warm season, the frequency of detection peaked on weekends and holidays (Fig. 2), and on those days, a distinct diurnal pattern was evident (Fig. 3).

Using the total industrial discharge of toluene reported in the National Pollutant Release Inventory (Environment Canada) (323 kg in 2000 used as an example), an expected concentration in the river was calculated. Using the simplifying assumptions that the industrial discharges were at a constant

Table 1. Number of analyses greater than menthod detection limit.

Analyte / Year	**mdl ppb**	**1996**	**1997**	**1998**	**1999**	**2000**	**2001****	**Total**
number of samples		8662	8642	8526	8567	8645	8609	51651
MTBE	0.10	0	28	1	1	0	0	30
Hexane	0.10	18	0	0	0	3	12	33
Chloroform	0.13	0	0	0	0	0	0	0
Cyclohexane	0.04	554	418	326	429	26	17	1770
Carbon tetrachloride	0.13	4	0	4	0	0	2	10
Benzene	0.05	310	436	402	374	396	165	2083
1,2-dichloroethane	2.9	0	0	0	0	0	0	0
Trichloroethylene	0.05	0	0	27	0	0	0	27
1,2-dichloropropane	0.09	200	78	0	0	0	0	278
Toluene	0.08	1695	808	1572	1748	1689	1050	8562
Perchloroethylene	0.08	38	0	24	15	22	25	124
Ethylbenzene	0.08	42	36	4	31	13	14	140
M+p-xylene*	0.16	196	130	100	208	134	99	867
o-xylene/styrene*	0.16	11	31	7	16	7	10	82
1,3-dichlorobenzene	0.09	0	0	0	3	1	0	4
1,3-diethylbenzene	0.12	0	0	0	0	1	0	1
1,2-diethylbenzene	0.09	2	3	1	25	0	0	31
tetraethyl lead	0.27	0	0	0	0	0	0	0
Total		**3070**	**1968**	**2468**	**2850**	**2292**	**1394**	**14042**

*analytes co-elute

**instrument problems reduced sensitivity for 3 months in 2001

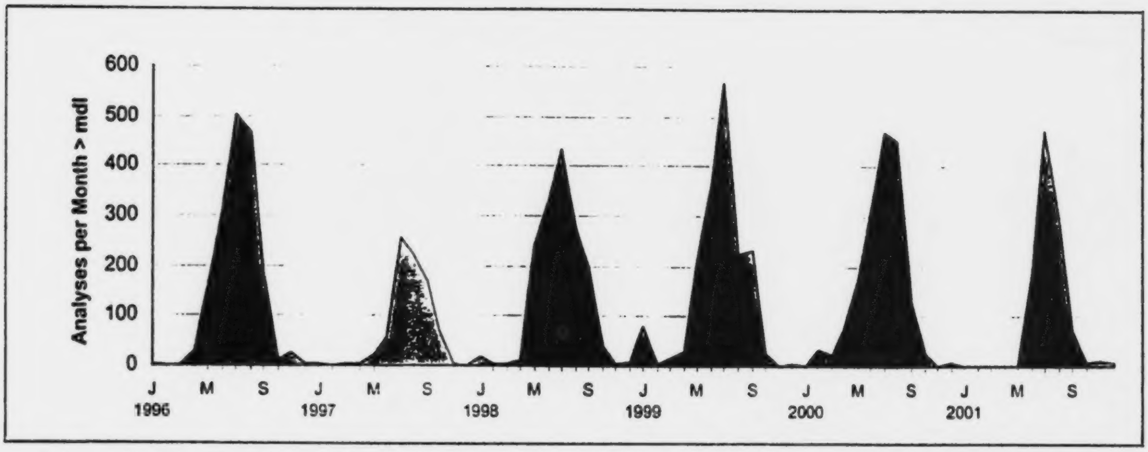

Figure 1. Seasonal variation in the frequency of detection of toluene at a fixed location in the St. Clair River. Analysis is hourly. Method detection limit is 0.08 ppb.

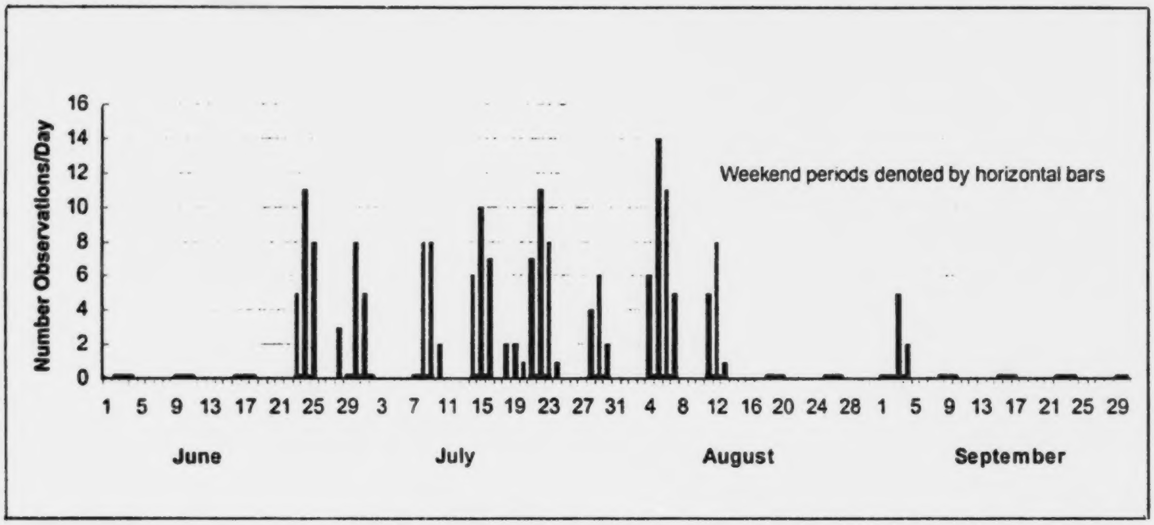

Figure 2. Weekly variation in the frequency of detection of toluene at concentrations above 0.20 ppb in the St. Clair River. Hourly analyses June through September, 2001. Weekend periods are denoted by horizontal bars along the date axis.

rate over the year, and were assimilated into five per cent of the river flow volume (St. Clair River Remedial Action Plan Team, 1991), the estimated concentration near the Canadian shoreline for 2000 was 0.039 µg/L. This value is consistent with the "not detected" reported about 85% of the time by the hourly monitoring.

Based on limited grab sampling, toluene was well mixed across and through the depth of the river on warm-weather weekends; and confined to the Canadian shore and/or below detection limits in cooler weather (Fig. 4).

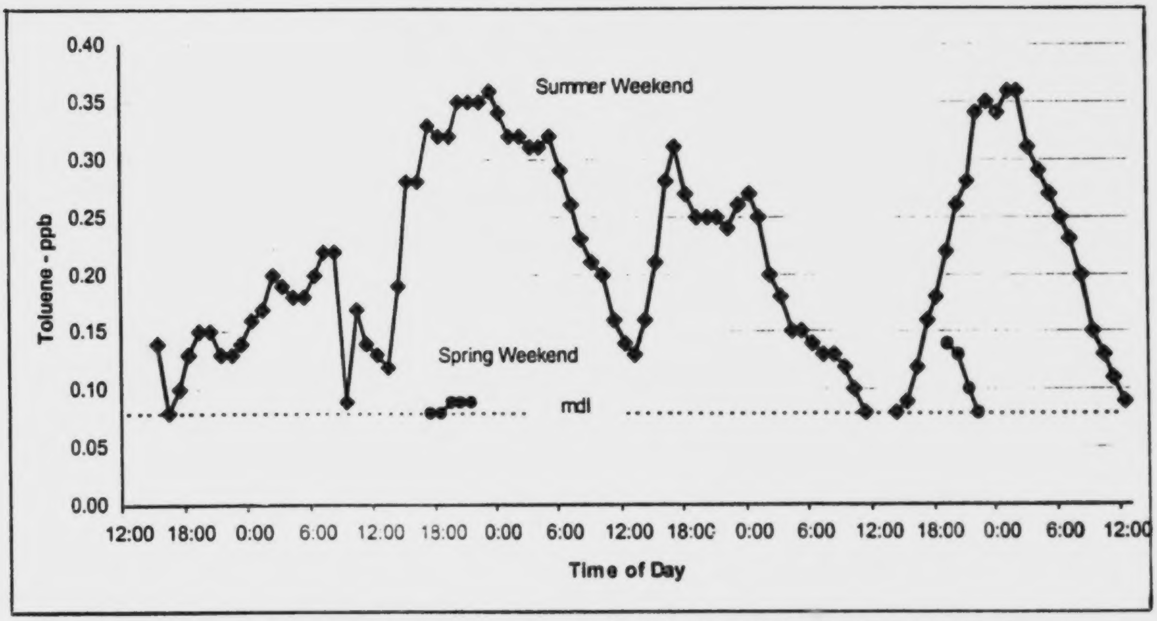

Figure 3. Diurnal variation of toluene concentrations in the St. Clair River on a summer weekend (Labour day weekend, 2001) and a spring weekend (April 23, 2002). Late evening peaks are consistent with current travel time from urban area at the Lake Huron head of the river to the the sampling point 18 km downstream.

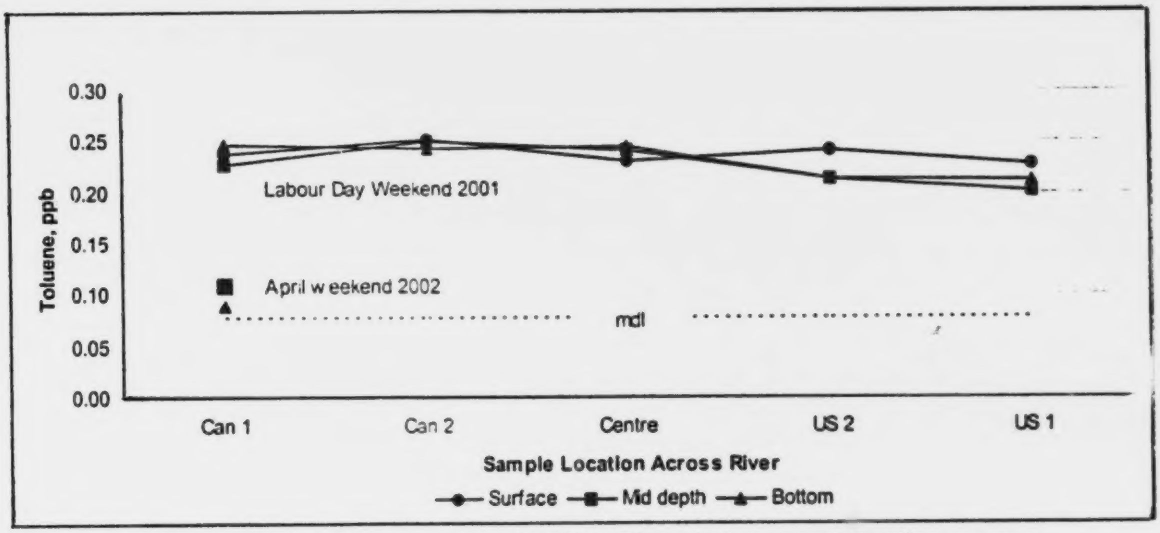

Figure 4. Transect sampling across the St. Clair River during a peak boating season weekend (Labour Day weekend 2001) and a spring weekend (April 23, 2002), form Canadian near-shore on the left (Can 1) to US near-shore on the right (US 1). Results at all locations except the Canadian near-shore location were below detection limits for the April 2002 transect.

Table 2. Estimation of non-industrial toluene load to St. Clair River Labour Day weekend, 2001.

Assumptions:

1. Toluene from non-industrial sources is well mixed torughout the river.
2. Diurnal pattern of hourly results at fixed sampling location is representative of full flow of river.

L_{pb} = load attributed to non-industrial sources, kg

where:

L_{pb} = load attributed to non-industrial sources, kg

C_{hri} = hourly concentration of toluene, kg/litre

F = 1.38 = Factor, average transect concentration/observed automated hourly concentration

L ind = 3.1 kg = estimated industrial load, per NPRI

n = 84 = hours of observation

A toluene load to the St. Clair River from non-industrial sources was estimated. Transect data for the summer weekend was averaged to obtain a single value assumed to represent the concentration of toluene in the full flow volume of the river at the time of sampling. The diurnal pattern reported by the hourly monitor was assumed to occur throughout the river. Using the hourly concentrations reported by the fixed monitor, a toluene loading of 186 kg over an 84 hour study period could be attributed to non-point sources, compared to 3.1 kg attributable to industrial point sources (Table 2).

Conclusions

Toluene concentrations in the St. Clair River were routinely below Ontario's Provincial Water Quality Objective and Drinking Water Standard; no measurable environmental or health impacts would be expected at the concentrations reported.

Seasonal, weekly and diurnal observations of toluene were consistent with peak periods of pleasure boat operation on the river. Annual toluene load to the St. Clair River due to pleasure boat operation was estimated to be three times the industrial contribution. Daily contributions of toluene attributable to boat operation during peak periods were one to two orders of magnitude greater than industrial loadings.

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Amphibian and Wildlife Toxicology

Session Co-chairs: J.E. Elliott and C.A. Bishop

Methods for Culturing and Toxicity Testing Using North American Amphibian Species: Report of a Workshop on the Standardization of Methods. B.D. Pauli and L.S. Benedetti. Environment Canada, National Wildlife Research Centre, Hull, QC.

Amphibians are considered to be sensitive indicators of environmental pollution, yet toxicity tests with amphibian species are not required in any Canadian regulatory programs. One of the reasons for this is a lack of standard methods for toxicity tests with native amphibians. Lack of standard methods has also hindered the acceptance of data from amphibian toxicity tests in assessments of toxic substances. We are attempting to standardize the methods used for culturing and toxicity testing with native amphibians using the Northern Leopard Frog, *Rana pipiens*, as the model species. We are employing both expert consultation (including convening a workshop) and laboratory experiments in this process. One of the first requirements identified is for a reliable supply of embryos for use in toxicity tests. We recommend that embryos come from in-house breeding colonies rather than being collected from field populations or purchased from commercial suppliers. This requires the establishment of standard protocols for breeding Northern Leopard Frogs. In addition, standard diet and holding conditions for all life stages must be established. Recent research efforts in various laboratories have identified a diet that produces stable and repeatable Leopard Frog tadpole growth rates, and some of the environmental cues necessary to stimulate breeding. Toxicity endpoints also require further research – while tadpole effects endpoints are fairly well established, more research is required into appropriate endpoints to assess in animals that have been taken through metamorphosis.

Standardization of an Amphibian Toxicity Testing Methodology: Our Progress and Priorities for Test Method Development. R.P. Scroggins[1] and A.N. Edginton[2]. [1]Environment Canada, Method Development and Applications Section, Ottawa, ON; and [2]Department of Environmental Biology, University of Guelph, Guelph, ON.

Environment Canada is embarking on a multi-year project to develop a standardized method for culturing and testing using a native Canadian amphibian species. The first phase of the method standardization process was to review current culturing and toxicity testing methodologies and to recommend further focused research that must be undertaken before a standard test methodology could be prepared. This review document, completed in October 2001, helped direct the discussion at a workshop on amphibian toxicity testing. This January 2002 workshop was attended by 40 individuals including experts from government, industry and academia.

Three working groups were formed at the workshop to provide a focused forum in which to discuss the key elements of a standard method, more specifically, amphibian culturing, toxicity testing methodology and relevant endpoint measurement. In the toxicity testing working group, consensus was reached on the scope of the standard method and recommendations for future research were identified. First, a decision was made that the northern leopard frog, *Rana pipiens*, be the species around which the methodology should be standardized. Second, it was recognized that current published testing procedures on amphibian early life stage toxicity testing do not fulfill the needs of a Canadian method, either due to the non-specificity of procedures or because test species used are not relevant to the Canadian environment (i.e., *Xenopus*). Third, it was recommended that the standard method include two test options: [1] an embryo exposure, and [2] a short-term chronic exposure encompassing embryonic to late larval stages. Further discussions revolved around procedure type (flow-through vs. renewal), water quality requirements, statistical issues, validity criteria and the importance of the development of a laboratory induced breeding protocol for *R. pipiens*. Environment Canada is planning a research program to generate the data needed to support methodology decisions that must be made to develop a standardized testing method for toxicity testing with early life stages of amphibians.

The Amphibian Sperm Inhibition Toxicological Test Method. J.R. Christensen[1], C.A. Bishop[2], J.S. Richardson[1] and J.E. Elliott[2]. [1]Forest Sciences Centre, University of British Columbia, Vancouver, BC; and [2]Environment Canada, Canadian Wildlife Service, Delta, BC.

The Amphibian Sperm Inhibition Toxicological Test (ASITT) method is fast, simple, cost-effective, and has the potential to contribute valuable information to a growing database of toxicological endpoints used to evaluate health risks. ASITT measures the effects of environmental contaminants on various *Xenopus laevis* sperm kinematics and sperm motility phases. The effects of pH, osmolality and Zn on sperm motility are discussed here. pH 7.0 displayed the highest percent motility of the entire range of pH's tested (5.5 to 7.8). Osmolalities (0 to 113.25 mosmol/L) caused a significant quadratic dose response with percent total motile and percent progressives. The 56.625 mosmol/L treatment had the highest percent motility and highest velocities. Seven concentrations of Zn were used in this research (0 to 1417 µg/L). Increasing Zn caused a significant decrease in percent motility in a linear dose response, where motility reached 64% of controls at 1417 µg/L. Percent progressives also decreased in a significant linear dose response. Previous research has shown that a decrease in sperm motility negatively affects fertilization success in amphibians. *X. laevis* sperm motility is very sensitive to the aquatic environment and could act as a sensitive water quality indicator. This test is in very preliminary stages and more work in this area may lend weight to ASITT's efficacy and usefulness as a standardized laboratory method for toxicity testing.

Environmental Occurrence of Lindane: Potential Effects on Anurans. K.C. Serben[1,2] and D.J. Forsyth[3]. [1]BC Research Inc., Vancouver, BC; [2]Toxicology Centre, University of Saskatchewan, Saskatoon, SK; and [3]Environment Canada, Canadian Wildlife Service, Saskatoon, SK.

Exposure to pesticides is a possible cause of global amphibian declines and increased occurrence of malformations in frogs. Pesticides can enter breeding ponds through runoff from agricultural cropland, direct spray, or atmospheric deposition. Lindane is an organochlorine pesticide that is used in North America as a seed treatment for a variety of crops. Significant quantities of lindane can be lost from the soil through volatilization to the atmosphere where it can be transported and deposited to adjacent water bodies or untreated land. Its high vapor pressure and resistance to photodegradation also allow it to remain in the atmosphere for long periods where it can be carried to northern pristine environments. Environmental groups have called for a complete ban of lindane, due to its persistence in the environment, its ability to bioaccumulate, and its potential as an endocrine disrupter. This paper discusses current uses of lindane, its distribution in the biosphere, and concentrations in soil, water, and air. The effects of lindane on anurans are described and related to tissue concentrations, when available. The exposure and effects data are compared in a hazard assessment to determine if environmental concentrations have the potential for adverse effects in anurans.

Carbamate Pesticide Toxicity to Salmon Olfaction: Effects and Potential Mechanisms. H.E. Jarrard, R. Lee, K. Delaney and C.J. Kennedy. Department of Biological Sciences, Simon Fraser University, Burnaby, BC.

An emerging issue of concern for endangered salmon populations is the toxicity of sublethal pesticide exposures. Pesticides can induce physiological/behavioral changes that impair survival and ecological fitness. We are interested in carbamate pesticide effects on salmon olfaction, as it is central to salmon life histories. Recent studies suggest that numerous pesticides impair salmon olfaction. This impairment is believed to occur through inhibition of acetycholinesterase (AChE), yet ACh has not been described in the salmon olfactory system. We are examining carbamate pesticide effects and mechanisms of toxicity through neurophysiology (to describe exposure effects on olfactory receptor neurons [ORNs]) and immunohistochemistry (to localize ACh in the olfactory system).

Neurophysiological impairment from carbamate exposure was examined in coho (*Oncoryhnchus kisutch*) using electro-olfactrograms (EOGs), a measure of ORN voltage potentials in response to odorant stimulation. EOG responses to L-serine delivery (10^{-5} M) were recorded from the olfactory epithelium (OE) before and after OE exposure to carbofuran (0.0001 to 2 mg/L), 3-iodopropynylbutylcarbamate (0.00048 to 0.048 mg/L), or mancozeb (0.022 to 2.2 mg/L). Significant reductions in EOG amplitude occurred with acute (30 min.) exposures to all carbamates examined (50% reduction: 0.02, 0.22, and 0.00048 mg/L respectively). ACh was localized with antibodies against choline acetyltransferase (ChAT catalyzes ACh production and is a marker for cholinergic neurons). Discrete OE regions exhibited α-ChAT-like immunoreactivity and contained numerous strongly labelled, mucous-secreting goblet cells. Surprisingly, the olfactory bulb appeared absent of ChAT. These results provide evidence that pesticide-induced olfactory impairment may be due to ACh-mediated hypersecretion of mucous in the salmon periphery.

Chlorinated Hydrocarbons in Fish and Osprey from Alpine and Reference Areas of British Columbia. J.E. Elliott[1], P. Shaw[2], M. Wayland[3], L.K. Wilson[1], G. Kardosi[1] and D.C.G. Muir[4]. [1]Environment Canada, Canadian Wildlife Service, Delta, BC; [2]Environment Canada, Vancouver, BC; [3]Environment Canada, Canadian Wildlife Service, Saskatoon, SK; and [4]Environment Canada, National Water Research Institute, Burlington, ON.

Eggs and nestling plasma of ospreys, and prey fish samples were collected between 1999 and 2001 from lakes and rivers situated at varying elevations and degrees of past chlorinated hydrocarbon input throughout British Columbia. Osprey eggs were analyzed immediately after collection and elevated DDE concentrations targeted for satellite tagging of adults. Blood samples were taken from chicks. A subset of study areas and nests were observed during the breeding season to identify osprey prey species. Satellite tracking (n=20) revealed wintering locations of ospreys, where we attempted to make further diet observations and collect fish samples. Highest concentrations of DDE (211 mg/kg wet wt whole carcass) in fish were found in samples of rainbow trout from Upper Jade Lake at 1817 meter elevation in Revelstoke Park. Toxaphene and PCBs were also found in Upper Jade Lake fish. Toxaphene, PCBs and chlordane compounds were found in all osprey samples. With the exception of an egg from a mid-elevation lake in Alberta, with 15 mg/kg DDE, all osprey eggs had <5 mg/kg DDE. Plasma sampling of chicks did not indicate any exceptional uptake of DDE in alpine drainages; chicks from the south Okanagan valley, an area of known DDE contamination, exhibited breeding ground uptake of DDE. Sampling of prey fish from both breeding and wintering grounds were consistent with the generally low levels of chlorinated hydrocarbons in osprey samples.

Migration within a Watershed Influences the Contaminant Profiles of American Dippers. C.A. Morrissey[1], L.I. Bendell-Young[1] and J.E. Elliott[2]. [1]Department of Biological Sciences, Simon Fraser University, Burnaby, BC; and [2]Environment Canada, Canadian Wildlife Service, Delta, BC.

From 1999 to 2002, an intensive study of a population of American dippers was established in a coastal watershed of southwestern British Columbia, Canada. Over 500 American dippers were individually color banded and followed to identify patterns of seasonal movement and its potential influence on contaminant burdens. The study revealed that the majority (~85%) of the dipper population seasonally migrated from the low elevation river to the higher elevation creeks while the remaining birds (~15%) remained on the river year-round. Through residue analysis of egg contents and breast feathers, we were able to detect a trend in contaminant profiles of resident and migrant American dippers. Total chlorinated hydrocarbons, polychlorinated biphenyls, and Hg were significantly higher ($p<0.0001$, $p<0.005$, $p<0.001$ respectively) in eggs from river residents compared to the creek migrants. The three most prevalent organochlorine compounds in dipper eggs, DDE, hexachlorobenzene, and trans-nonachlor, were all significantly higher on the river compared to those from the creeks. Additionally, feather HG ($p=0.068$) and feather Cd ($p=0.02$), but not feather Pb ($p>0.7$), showed higher mean concentrations in the river residents. These results emphasize the importance of understanding the ecology of the species to be able to correctly assess toxicological effects at the population level.

Blood Mercury Levels and Reproductive Success of Bald Eagles (*Haliaeetus leucocephalus*) Breeding in the Pinchi Lake Region of British Columbia, Canada, 2000 and 2001. S.A. Weech[1], A.M. Scheuhammer[2] and J.E.. Elliott[3]. [1]University of British Columbia, Vancouver, BC; [2]Environment Canada, National Wildlife Research Centre, Hull, QC; and [3]Environment Canada, Canadian Wildlife Service, Delta, BC.

An area along the Pinchi fault in central British Columbia is a known source of natural Hg in the form of cinnabar (HgS). In addition, in the past, Hg mining-related wastes were deposited directly into Pinchi Lake. To determine whether elevated Hg levels from this natural/mining-related source are adversely affecting bald eagle reproduction, eagles breeding on 5 study lakes (Pinchi, Tezzeron, Stuart, Great Beaver and Fraser) were monitored for nesting success during the summers of 2000 and 2001. Over the two seasons, nesting success (the total number of active nests found at the beginning of May that produced 8-week-old eaglets) was 63% on Pinchi compared to 78% on all other study lakes combined (no significant difference, z-test, p=0.743). In addition, 31 bald eagle chicks and 5 adult eagles were captured for blood sampling. Hg levels were significantly greater ($p<0.001$) in chicks from Pinchi (mean=0.62 μg/mL) compared to all other study lakes (combined mean=0.29 μg/mL). The two adults captured on Pinchi had 9.44 and 4.25 μg/mL of Hg in blood, with the remaining three eagles captured on Fraser, Stuart and Tezzeron averaging 3.93 μg/mL. For comparative purposes, blood Hg was also measured in 46 bald eagles captured or brought into rehab centers from various locations in British Columbia from 1993 to 2000. Blood Hg averaged 0.99 μg/mL (range=0.18 to 2.61 μg/mL, SD=0.50), much lower than the levels observed for Pinchi birds. It should be noted, however, that eagles from the Pinchi Lake region feed primarily on fish from a lacustrine habitat, whereas the feeding habits of the other eagles for which blood samples were available are not known. Different diets (e.g., aquatic vs. terrestrial) can significantly affect Hg exposure, and the levels of Hg in blood. Despite the high Hg level found in the one adult eagle from Pinchi, this bird appeared to be in excellent condition, with no evidence of abnormal behavior or lack of coordination, and successfully raised two eaglets that summer. Thus, while dietary Hg exposure, as measured by blood Hg concentrations, is greater in chicks and adults from Pinchi Lake, it does not appear to adversely affect eagle productivity.

Productivity and Chlorinated Hydrocarbons in Great Blue Herons (*Ardea herodias*) from Coastal British Columbia, 1977 to 2000. L.K. Wilson[1], J.E. Elliott[1], R.W. Butler[1], R. Norstrom[2] and M.L. Harris[3]. [1]Environment Canada, Canadian Wildlife Service, Delta, BC; [2]Environment Canada, Canadian Wildlife Service, Hull, QC; and [3]Lorax Environmental, Penticton, BC.

Great blue heron (*Ardea herodias*) eggs were collected from 23 colonies along south coastal British Columbia from 1977 to 2000 and analyzed for organochlorines (OC) and polychlorinated biphenyls (PCBs). Eggs from 21 rookeries were monitored from 1983 to 1998 for polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs). OCs and PCBs declined sharply in the late 1970s, after which there were minimal changes.

Eggs collected along the Fraser delta showed higher levels of most OCs compared to other colonies. Although the delta supports a long-standing agricultural economy, primary factors influencing OC levels in the delta colonies were driven by estuarine processes. Eggs from urban colonies contained higher levels of PCBs. The congener pattern did not differ from other colonies, except that colonies in Vancouver had a greater proportion of PCB 66, suggesting a local source of Aroclor 1242. Dominant PCDD/F congeners fell markedly in the early 1990s after pulp mills changed from molecular chlorine bleaching, and use of chlorophenolic wood preservatives and anti-sapstains was restricted.

Strong positive linear regression between prey fish and contaminants in heron eggs suggested that local dietary uptake was an important exposure route for herons. Toxic equivalent concentrations (TEQs) sufficient to produce embyrotoxicity in chicks were measured in eggs from 1985 to 1991 at some colonies. Despite reduction in PCDD/Fs, estimated TEQs remained elevated throughout the 1980s at some urban colonies due to PCB contributions. Productivity was variable over the study period; 71% of colony-wide failures occurred in colonies near pulp mills. However, predominant factors influencing productivity were probably disturbance (human, bald eagle) and loss/degradation of nesting habitat, not sublethal toxicity.

Fluctuating Asymmetry in Wood Frog Metamorphs Exposed to Lindane as Tadpoles in an Outdoor Microcosm Study. K.C. Serben[1,2] and D.J. Forsyth[3]. [1]BC Research Inc., Vancouver, BC; [2]Toxicology Graduate Student Program, Toxicology Centre, University of Saskatchewan, Saskatoon, SK; and [3]Canadian Wildlife Service, Environment Canada, Saskatoon, SK.

Measurement of fluctuating asymmetry (FA) has been promoted as an early warning biomonitoring tool to detect effects of stressors on populations prior to the manifestation of more obvious effects, such as a decline in population size. Few studies have used this endpoint in pesticide toxicity studies. Wood frog (*Rana sylvatica*) tadpoles were exposed to low concentrations of lindane throughout the larval period in outdoor microcosms. Four traits were measured six times on each metamorph: femur length, tibiofibula length, radio-ulna length, and eye-naris length. Signed (L-R) differences were converted to absolute FA estimates (|L-R|) for analysis of lindane treatment effects. Despite the large number of repeated measurements and the large sample size, the measurement error was high: overall percent measurement error (%ME) for each trait was 46% (femur length), 54% (tibiofibula length), 62% (radio-ulna length), and 83% (eye-naris length). The levels of FA, after factoring out %ME, were too low to detect any differences due to lindane treatment. While effects were observed on weight, hormone concentrations, and sex differentiation, no significant differences were observed with FA, indicating that this endpoint was not sensitive enough to serve as a biomarker of exposure to lindane in the wood frog.

Biodiversity and Cumulative Effects

Session Co-chairs: W.G. Landis and J.L. Parrott

Implementation of a Cumulative Effects Framework for Aquatic Ecosystems. M.G. Dubé[1], B.N. Johnson[2], G.W. Dunn[3], B.D. Johnston[1] and K.R. Munkittrick[4]. [1]Environment Canada, National Water Research Institute, Saskatoon, SK; [2]Environment Canada, Atmospheric Environment Branch, Regina, SK; [3]Environment Canada, Environmental Conservation Branch, Regina, SK; and [4]Department of Biology, University of New Brunswick, Saint John, NB.

Recently, we proposed a conceptual framework that integrates the strengths of stressor-based (S-B) and effects-based (E-B) approaches for aquatic cumulative effects assessment (CEA). One of the key challenges in implementing this framework is in quantifying existing environmental state using an E-B approach that is consistent with CEA as defined under the *Canadian Environmental Assessment Act* and manageable in the realm of existing environmental assessment practice. CEA by definition occurs over large geospatial and temporal scales and is a continuous process integrating environmental responses to multiple natural and anthropogenic stressors.

Under current EIA practice in Canada, CEA is site and project specific. Our objectives are to: [1] describe a process for conducting a regional and continuous E-B assessment of aquatic systems while accommodating the site-specific nature of current EIA practice, [2] describe a GIS-based software tool under development in Pacific and Northern Region of Canada to implement the E-B approach, and [3] describe how this E-B information can be used in the S-B component of the CEA framework. Our approach recognizes that a wealth of aquatic data exists in Canada, and for many of these data sets, guidelines, benchmarks, and effect definitions have been established and applied by their respective agencies.

The Application of Canadian Water Quality Index (CWQI) in the Mackenzie River Basin. A. Lumb[1], D. Halliwell[2] and T.C. Sharma[3]. [1]Environment Canada, Ecological Monitoring and Assessment Network Coordinating Office, Burlington, ON; [2]Environment Canada, Meteorological Service of Canada, Yellowknife, NT; and [3]102-240, Markland Drive, Etobicoke, ON.

The Canadian water quality index (CWQI) has been applied as a tool to monitor the changes in water quality at various sites in the Mackenzie–Great Bear sub-basin, NT. The water quality at these sites has been evaluated by using two modes of threshold values based on the CCME guidelines and the site-specific values that are determined by the statistical analysis of the data. The CCME based objective functions were applied on 8 different protocols, each representing a typical scenario to evaluate the water quality employing CWQI Macro. The site-specific values were applied on the set of variables meant for overall use of water. The site-specific objective functions were constituted in three combinations of the statistics of the data series, resulting in 3 additional protocols.
The site-specific values were applied on the set of variables meant for overall use of water as indicated in protocol 1. This evaluation revealed: [1] the high turbidity in Mackenzie River Basin always attributed towards lowering the water quality and can be controlled by adopting appropriate watershed management strategies, [2] the water quality had deteriorated in the 90's due to excessive presence of trace metals that can be attributed to natural and anthropogenic sources. Several mines were decommissioned in this area around that period which might have encouraged uncontrolled runoff from that area, and [3] the categories of water quality evaluated by CWQI based on site-specific and CCME guidelines did not provide conclusive results therefore no preferential use of either of them can be emphasized. However, site-specific thresholds allow the use of more water quality variables. This new approach using different protocols does assist in identifying the problematic variables/parameters that may be contributing towards lowering the quality of water and this information is of great value for the users (public), water supplier (municipalities and city councils) and the planners and policy makers.

Effects of Experimental Additions of a Synthetic Estrogen on a Freshwater Food Web. K.A. Kidd, V.P. Palace, P. Blanchfield, R.E. Evans, K. Mills, C. Podemski, A. Salki, M. Paterson and D. Findlay. Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, MB.

Effects of a potent hormone mimic are currently being examined in fish populations and lower-trophic-level organisms from a lake experimentally treated with the synthetic estrogen ethynylestradiol (EE2). EE2 was continuously added to Lake 260, a small pre-cambrian shield lake at the Experimental Lakes Area in northwestern Ontario, for 5 months in both 2001 and 2002. To date, male fish from the lake have up to 9000 times higher concentrations of the egg yolk precursor vitellogenin than was detected in fish captured from the same lake prior to the EE2 additions, or when compared to fish collected from reference lakes. In addition, some males displayed widespread fibrosis and inhibited development of the testis, as well as testis-ova. These responses at the biochemical and tissue level are being linked to effects on populations including abundance, growth rates and sex ratios. While no effects have been observed on algal or microbial communities, some zooplankton species in EE2-amended Lake 260 had fewer males and females that produced fewer eggs when compared to baseline data. Results indicate that low concentrations of a potent estrogen mimic can have adverse impacts on both vertebrates and invertebrates in aquatic ecosystems.

The Wilmot River: Anatomy of a Murder. K.L. Gormley, K.L. Teather and D. Guignion. Department of Biology, University of Prince Edward Island, Charlottetown, PE.

The Wilmot River in Prince Edward Island is one of the provinces longest rivers at 78.3 km and has a watershed area of approximately 166 km^2. Despite being situated in the heart of one of the most concentrated potato farming regions on the Island, the Wilmot contained large populations of both brook trout (*Salvelinus fontinalis*) and rainbow trout (*Oncorhynchus mykiss*). In July 2001, three sites along the river were sampled and populations estimates were (for 100 sq. m): Murphy's - 105 brook, 0 rainbow; Route 109 - 227 brook, 21 rainbow; Route 110 - 93 brook, 21 rainbow. On 10 July, 2002, sediment from a neighbouring potato field that had recently been treated with the insecticide azinphos-methyl, entered the stream after a heavy rain between Murphy's and Route 109, resulting in high fish mortality. Eight days later, a second runoff event occurred between the sites at Route 109 and 110, again, with large numbers of fish killed. Subsequent population estimates, taken within a few days of these events, revealed that [1] brook trout suffered more serious declines than did rainbow trout, and [2] younger age classes may have been more susceptible than higher age classes. Since the second fish kill, other sampling sites have been added so that we can monitor changes in community structure during the rest of the summer and over the next few years.

Design Considerations for an Ongoing Cumulative Effects Monitoring Program for the Uranium Mining Industry. T. Moulding and N. Dyck. Saskatchewan Environment, La Ronge, SK.

In response to recommendations made during the 1993 Joint Federal/Provincial Panel on Uranium Developments in Northern Saskatchewan, the Governments of Saskatchewan and Canada established the Uranium Mining Cumulative Effects Monitoring (CEM) Program in 1994. The program is directed by representatives from the provincial and federal governments, the uranium mining industry, and the University of Saskatchewan. In April 2001, it was decided that the information gathered should be compiled and analysed in order to ensure that the program was fulfilling its objective. As a result, a comprehensive report including statistical analysis of the data gathered was commissioned. In the preparation of the comprehensive report, statistical analysis of the data revealed deficiencies in the sampling program. Most of the deficiencies were the result of program limitations of budget, time, and the size of the study area. In order to address these deficiencies, the CEM program is now undergoing refinement. The objective of the refinement will be to ensure that the program will be able to detect actual effects in a scientifically defensible manner and that effects will be determined to an agreed upon level of statistical significance.

Future design considerations will include: modifications to the sampling program to ensure that confidence levels for statistical analysis will be met; focusing of resources on areas identified in the program as being the most likely affected; increased cooperation of industry and the use of industry data to augment the database; and ensuring that the program continues to fulfil the expectations of the stakeholders and the original mandate. The first test of the refinement efforts will be a sampling program scheduled for September 2002 in the area of the Key Lake Mine.

Dealing with the Data – Using Biotic Indices as Environmental Indicators. G.P. Thomas and K.A. Munro. G3 Consulting Ltd., Burnaby, BC.

Environmental effects monitoring for specific industries and other environmental studies include biological sampling and taxonomic work. Algae, invertebrates and fish integrate and reflect effects of the physical and chemical environment. Much is known about tolerance to specific contaminants. What do we do with all the expensive species distribution data collected? Unless we are specialists, we may feel overwhelmed by the mass of data, while suspecting there is a wealth of information to be mined. This presentation discusses strengths and limitations to use of indices. Many indices have been developed, from the Shannon-Weiner diversity index to Karr's Index of Biotic Integrity. A workable index should be based in ecological reality, be multimetric with many links to underlying processes, and integrate the data without oversimplifying. It should be descriptive, prescriptive and adaptive. Biotic indices provide tools for translating data into information, then communicating, particularly to decision makers and the public. They provide a "value-added" service, using data already collected and, occasionally, additional analyses, such as fitness indicators and presence of deformities. The approach helps provide the "so what?" of monitoring and assessment. Results can be combined with various statistical approaches and incorporated into weight-of-evidence analysis and cumulative impact, river health and risk assessments.

Cumulative Effects: Climatic Influences on Metal Uptake and Stress Physiology in Land-Locked Arctic Char. D.A. Bright[1], G. Koeck[2] and C. Doblander[2]. [1]Applied Research, Royal Roads University, Victoria, BC; and [2]University of Innsbruck, Innsbruck, Austria.

This study, based on annual sampling in the Canadian Arctic Archipelago from 1997 to 2002, aimed to elucidate the interactions between intra- and inter-annual variation in lake water temperature, the uptake in non-anadromous Arctic char (*Salvelinus alpinus*) populations of metals (Cd, Pb, Zn), induction of biochemical stress indicators (metallothionein, glutathione, glutathione disulfide, riboflavin, Vitamin-C) and energy status (liver and circulating glycogen levels). In 1998, higher than normal summer-time lake water temperatures and a longer ice-free period were accompanied by a clear physiological response of the fish studied. Both the concentration of metals (Cd, Zn) in fish liver or kidney and metal-induced stress levels were significantly higher in August of 1998 when compared to the same period during the colder years 1997, and 1999 to 2001. Concentrations of Cd in the liver were positively correlated with the mean summer air temperatures at the sampling site. Results will be explored in terms of lake temperature controls on metal bioaccumulation, otolith microchemistry, bioenergetics and the fitness of high latitude non-anadromous fish populations. The results provide a compelling example of how biological changes in the face of rapid climate change may be extremely hard to predict, and often counter-intuitive.

Health Status of Blue Mussel Populations Collected from a Canadian Harbour during a Reproductive Cycle. J. Hellou[1], P. Yeats[1], S. Steller[1] and F. Gagné[2]. [1]Department of Fisheries and Oceans, Bedford Institute of Oceanography, Dartmouth, NS; and [2]Environment Canada, Centre Saint-Laurent, Montreal, QC.

Mytilus edulis were collected inter-tidally from three locations in Halifax Harbour, on five occasions during the summer of 2000. They were analysed for organic and metal contamination in whole soft tissue of males, in gonads of females and in the rest of females' soft tissue. Bioindicators of health included lipid content, condition and gonad indices, sex ratio as well as vitellogenin-like proteins. Twice as many males as female mussels were collected from a downtown site (M8) close to numerous raw sewage effluents and a naval dockyard. These mussels also displayed the highest levels of polycyclic aromatic compounds (PACs), coprostanol, Ag and Sn.

Males from M8 had a high lipid content and females had a delayed production of vitellins. Female mussels were more abundant in an area outside the industrialized part of the harbour with higher marine traffic (M14), but were not otherwise easily distinguished. The lowest variability in biomarkers was observed at a site in a mostly residential arm of the harbour (M12) expected from an earlier investigation to be more pristine. These mussels had the lowest condition indices and PCB concentrations, and low but similar levels of lipids, PACs and coprostanol to those at M14. They also displayed the highest concentrations of Cd, Cu, Pb and Hg and females had the highest gonad indices early in the season. These observations indicate that the health of mussels in Halifax Harbour is affected by complex interactions of harbour and urban contaminants.

Ecological and Ecotoxicological Studies on the Inter-tidal Amphipod, *Corophium volutator*, in the Upper Bay of Fundy, Gulf of Maine. P.G. Wells[1], M. Trites[2], I. Kaczmarska[2], P. Hicklin[3], J. Hellou[4], D.J. Hamilton[5], K.G. Doe[6], M. Brylinsky[7] and M.A. Barbeau[5]. [1]Environment Canada, Canadian Wildlife Service, Dartmouth, NS; [2]Department of Biology, Mount Allison University, Sackville, NB; [3]Environment Canada, Canadian Wildlife Service, Sackville, NB; [4]Department of Fisheries and Oceans, Bedford Institute of Oceanography, Dartmouth, NS; [5]Department of Biology, University of New Brunswick, Fredericton, NB; [6]Environment Canada, Moncton, NB; and [7]Acadia Centre for Estuarine Research, Acadia University, Wolfville, NS.

The Corophium working group (WG) is a research-oriented group of the Bay of Fundy Ecosystem Partnership (BoFEP; see www.auracom.com/~bofep) focusing on the biology, ecology and ecotoxicology of this Gammaridean amphipod. *Corophium* occurs on mud flats in the upper Bay and is a key food for migrating shorebirds. The WG maintains a bibliography and collection of key papers and reports on Corophium species. Brylinski is data mining the literature to identify and verify variables influencing the distribution and abundance of *Corophium.* Hamilton's recent studies, including exclosure studies at Avanport Beach and surveys of several mudflats in the upper Bay, have elucidated some of the primary biotic and abiotic factors that control abundance of Corophium and other inter-tidal species, including shorebirds. Research by Barbeau has concentrated on the seasonal population dynamics of Corophium at beaches on both sides of the Bay. Kaczmarska focuses on the inter-tidal diatoms and their ecology, as they are a primary food for *Corophium.* Hicklin continues yearly sampling and studies in Shepody Bay in association with the predation pressures induced by the migratory shorebirds. Doe is using *Corophium* as a model organism for toxicity studies, currently investigating the effects of Orimulsion and No. 6 fuel oil. Hellou is interested in the bioavailability, in terms of fate and effects of contaminants, especially PACs, to *Corophium.* Wells and team have been characterising the sediments *Corophium* lives in, using Microtox® SPT test, and have an extensive data set across the upper Bay.

Assessment of Water Contaminants and the Microbial Potential in Urban Aquatic Environments. G. Krauss[1], K.R. Sridhar[2], D. Jain[1], M. Möder[3], G. Strauch[4] and D. Schlosser[1]. [1]Groundwater Microbiology Junior Research Group, [3]Department of Analytical Chemistry, and [4]Department of Hydrogeology, UFZ Centre for Environmental Research Leipzig-Halle, Halle/S., Germany; and [2]Department of Biosciences, Mangalore University, Mangalore, India.

As yet widely ignored chemical water contaminants related to urban activities now are gaining increasing attention because of their risk potential for human health and the environment. In a first sampling campaign we have assessed water and sediments of the Saale river at different sampling sites in and around the city of Halle/Germany. We could detect varying concentrations of 4-nonylphenol, bisphenol A, polycyclic musk compounds, phthalates, and PAHs as indicators for urban pollution. Microbial activity is one of the major parameters affecting the environmental fate of such contaminants and may lead to the formation of metabolites with an often uncertain ecofate. The microbial community of the test sites was characterized by total counts of different ecophysiological groups of bacteria and water-borne fungi. Specific isolation techniques and monitoring of extracellular enzyme activities related to the degradation of organic compounds implicated aquatic hyphomycetes and other water-borne fungi as promising candidates for microbial metabolism models. Furthermore, pathogenic agents (classical pathogens or potentially emerging new pathogens) have to be controlled to ensure the sustainable usage of water resources. To indicate faecal pollution, we have also assessed cell numbers of *E. coli* and total coliforms throughout the sampling sites.

Genomics and Molecular Techniques

Session Co-chairs: C.C. Helbing and T.W. Moon

Genomics Research on Atlantic Salmon Project: Its Application to Toxicology and Environmental Assessment. B.F. Koop[1], B. Hoyheim[2] and W. Davidson[3]. [1]Department of Biology, University of Victoria, Victoria, BC; [2]Norwegian school of Veterinary Medicine, Oslo, Norway; and [3]Department of Molecular Biology and Biochemistry, Simon Fraser University, Burnaby, BC.

The Genomics Research on Atlantic Salmon Project (GRASP), funded by Genome BC, and a corresponding initiative in Norway have been designed to provide the foundation for understanding the genome of Atlantic salmon. It should be noted, however, that genomic information gained from Atlantic salmon will be applicable to other salmonid species. Moreover, this information will be useful for more than just the development of aquaculture. It will benefit conservation and enhancement of wild stocks, commercial harvesting through the identification of specific stocks, the maintenance of lucrative sports fisheries, enable fundamental scientific questions concerning the evolution of salmonid genomes to be answered and it will facilitate monitoring the expression of genes and proteins in a wide variety of natural and altered environments.

The specific aims of the Atlantic salmon genome projects are: [1] to tie together the linkage map based primarily on microsatellite markers with the physical map based on BAC contigs and position these on the chromosomes, [2] to locate genes of known function on the physical map, to gain a better appreciation of the structure and function of constituents of the immune system, and to compare specific regions of the Atlantic salmon genome in order to understand how a duplicated genome reorganizes itself, controls sex-determination, and is related to the genomes of other vertebrates, and [3] to examine gene expression at the transcriptional level and the translational level in several tissues under different conditions, and to identify molecules induced by physiological responses to stress, acclimatization, exposure to pathogens and exposure to natural and introduced chemicals. More than 30 cDNA libraries have been constructed from a wide variety of tissues and different developmental stages. At the end of May 2002, more than 40,000 reads of the 3' ends of these expressed sequence tags (ESTs) have been completed and these constitute approximately 17,000 contigs or independent gene products. Preliminary analysis of these data reveals the presence of many duplicated gene products.

A BAC library has been constructed and 313,000 clones with an average insert size of 170,000 to 190,000 base pairs have been selected for DNA fingerprinting and contig construction at the BC Cancer Agency Genome Sciences Centre. This represents a 15 fold coverage of the genome. A microarray with 1,000 genes represented is being prepared and it is anticipated that this will provide salmonid researchers with the opportunity to initiate expression studies. This presentation will give an update on the status of the genomics project.

Molecular Responses to Nonylphenol Exposure in Trout Hepatocytes: A cDNA Microarray Study. S. Wiseman and M.M. Vijayan. Department of Biology, University of Waterloo, Waterloo, ON.

The ability to simultaneously monitor the expression of thousands of genes makes cDNA microarrays an extremely powerful tool in physiology and environmental toxicology. In collaboration with Drs. Tom Mommsen, Graham van Aggelen and Caren Helbing, we have developed a rainbow trout-specific cDNA microarray incorporating a battery of 150 genes involved in cellular physiology, including growth, immune responses, endocrine function, metabolism and reproduction. In this study, using the trout cDNA microarray, our objectives were [1] to compare and contrast the gene expression changes seen with nonylphenol (NP) from that seen with estradiol, and [2] to determine whether NP had an impact on other endocrine axes. To this end, we used hepatocytes in primary culture as a model system to characterize the utility of the microarray, especially because the *in vitro* system allowed for parallel treatment comparisons, thereby reducing inter-fish variability inherent with *in vivo* studies.

Our results suggest that a suite of genes are either up- or down-regulated in response to NP. Some of these changes were common to both the NP and estradiol treated hepatocytes. However, NP exposure resulted in the expression of several genes that were unique and not observed in the estradiol group. Moreover, the effect of NP was not limited to genes involved with only the endocrine axes, but also included many metabolic and stress-related genes. We are currently using quantitative real-time PCR to validate the magnitude of NP-mediated gene expression changes evident with the trout microarray. This study was funded by NSERC, Canada. We thank Ms. Roula Raptis and Ms. Jana Malhotra for their assistance.

Exposure to the Herbicide Acetochlor Alters Thyroid Hormone-Dependent Gene Expression and Metamorphosis in *Xenopus laevis*. D. Crump[1], K. Werry[1], N. Veldhoen[1], G.C. Van Aggelen[2] and C.C. Helbing[1]. [1]Department of Biochemistry and Microbiology, University of Victoria, Victoria, BC; and [2]Environment Canada, Pacific Environmental Science Centre, North Vancouver, BC.

A growing number of substances released into the environment function as disruptors of critical, normal endocrine mechanisms in a wide range of vertebrates. Relatively little is known about the effects and identities of endocrine disrupting chemicals (EDCs) that target thyroid hormone (TH) action, particularly at the cellular level. Frog tadpole metamorphosis is completely dependent upon TH which has led to the suggestion of a metamorphosis-based assay for screening potential EDCs. A major mechanism of TH action is the alteration of gene expression via hormone-bound nuclear receptors (TRs).

To assess the gene expression profiles in the frog model, we have designed a novel multispecies frog cDNA microarray containing over 400 different gene sequences. Specific transcript levels were also determined using quantitative real-time polymerase chain reaction analysis. Recently, the pre-emergent herbicide acetochlor was shown to accelerate 3,3',5-triiodothyronine (T_3)-induced forelimb emergence and increase TRβ mRNA expression in Ranid tadpoles. Herein, we show that T_3-induced metamorphosis of *Xenopus laevis*, a Pipid species commonly used in the laboratory, is accelerated upon acute exposure to an environmentally relevant level of acetochlor. The morphological changes observed are preceded by alterations in gene expression profiles detected in the tadpole tail.

Gene Expression Patterns in Fish Exposed to Compounds that Mimic Estrogen. N.D. Denslow[1], P. Larkin[1], T. Sabo-Attwood[1], M. Hemmer[2] and L.C. Folmar[2]. [1]Department of Biochemistry and Molecular Biology, University of Florida, Gainesville, FL; and [2]USEPA, NHEERL, Gulf Ecology Division, Gulf Breeze, FL.

We have developed estrogen-sensitive gene arrays to measure changes in gene expression in sheepshead minnows and largemouth bass exposed to anthropogenic chemicals that mimic estrogen. The *in vivo* exposures, which realize the full physiological response in fish, result in changes at the molecular level that can be deciphered by gene arrays. Sheepshead minnows were exposed to environmental concentrations of strong estrogens: 17-*β*-estradiol, 17-α-ethynylestradiol, diethylstilbestrol, and weak estrogens: methoxychlor, *p*-nonylphenol and endosulfan in a flow-through format. While a general estrogenic response pattern was observed for all the exposures except endosulfan, the response of some genes varied depending on the compound used, suggesting that contaminant-specific patterns of gene regulation may exist. The chips were useful to measure dose-dependent changes in gene induction for fish treated with 20 ng/L to 1000 ng/L ethinylestradiol. Largemouth bass were exposed to 17-beta-estradiol, *p*-nonylphenol and 1,1-dichloro-2,2-bis (*p*-chlorophenyl) ethylene (*p,p'*-DDE) by IP injection. Again, we observed similar contaminant-specific up-regulation of gene expression in fish exposed to 17-beta-estradiol and *p*-nonylphenol. Initial experiments suggest that *p,p'*-DDE has different induction effects in males and females; where known estrogen sensitive genes are up-regulated in males while the same genes are down-regulated in females. Overall, this research suggests that a better understanding of the consequences of exposure can be obtained from monitoring multiple genes at one time.

Toxicogenomics and the Environment : Emerging Toxicological Rainbow Trout Method for Measurement of Deleterious Effects. H.L. Booth, J.B. Bruno and G.C. van Aggelen. Environment Canada, Pacific Environmental Science Centre, North Vancouver, BC.

The rainbow trout test has and continues to serve us well. John B. Sprague's pivotal 1972 paper and Environment Canada's 1990 methods manual both prescribed how to conduct a fish bioassay and have historically made us use death as the definitive end-point. For the last 30 years the rainbow trout 96 hour LC50 test has been the "gold standard" for the measurement of acute lethality of aqueous discharges into the environment. The Federal Government, under the *Fisheries Act*, and several Provincial Governments, each with their own legislation, have included the requirement of using the pass/fail compliance test of greater than 50% survival, in 100% concentration, after 96 hours of exposure as their chief means of protecting aquatic life. As a consequence of public environmental awareness and industries striving to meet both various Federal and Provincial regulatory requirements, industries have, for the most part, achieved non-toxic effluents as measured by the traditional pass/fail bioassay. Recent research has shown that non-acutely lethal effluents are capable of causing endocrine disrupting effects in aquatic organisms. With the emerging discipline of toxicogenomics, we now have the ability to measure molecular level acute and chronic deleterious effects in both *in-situ* and laboratory settings. By using microarrays to evaluate toxicant induced gene expression changes we have added a more sensitive and relevant end-point measurement for deleterious effects. For the past three years our collaborative group has been developing rainbow trout and frog cDNA microarrays. We are currently at the stage of testing effluents, receiving waters, and other suspected EDC sources with the view of establishing a standardized application of the microarray technology using the rainbow trout test platform.

Expression of a Glutathione *S*-transferase that Provides Protection Against Oxidative Injury in Largemouth Bass Liver. E.P. Gallagher, A.M. Doi, R.T. Pham and E. Hughes. Department of Physiological Sciences, and Center for Environmental and Human Toxicology, University of Florida, Gainesville, FL.

A number of environmental pollutants, including certain pesticides, metals, and components of complex mixtures are capable of causing oxidative injury in aquatic species. We are currently investigating the role of key oxidative defense enzymes in protecting against the sublethal effects of chemical exposure in largemouth bass (*Micropterus salmoides*), a higher order predatory game fish. Enzymatic studies indicate that bass liver glutathione *S*-transferases (GSTs) can very rapidly and efficiently detoxify 4-hydroxy-2-nonenal (4HNE), a common mutagenic and cytotoxic aldehyde produced during exposure to pro-oxidant pollutants. To identify important GST isozymes involved in 4HNE detoxification, we isolated a full-length GST cDNA of 957 base pairs (bp) in length, containing an open reading frame of 666 bp and encoding a polypeptide of 222 amino acids. The recombinant expressed protein showed high catalytic activity towards 4HNE, and HPLC-GST subunit analysis followed by protein sequencing demonstrated that the isolated GST subunit constitutes the major GST protein in bass liver. GST proteins homologous to this bass isozyme were found in other fish species, including plaice (*Pleuronectes platessa*), fathead minnow (*Pimelphales promelas*), and European flounder (*Platichthys flesus*), but not in mammals. In summary, the presence of a highly expressed GST isozyme in bass and evolutionarily divergent species indicates the conservation of an important protein involved in protection against oxidative damage in several aquatic species. Our results also underscore the importance of characterizing specific biotransformation isozymes when assessing the ability of fish to detoxify toxicologically relevant substrates through multigenic biochemical pathways. Modulation of bass liver GST expression by estrogenic compounds will also be discussed. Supported by NIH P42 ES07375.

Proteomic Analysis of Fish Cell Lines and Potential Applications in the Development of Biomarkers of Aquatic Toxicity. L.E.J. Lee, S. Willfang, M.S. Allen and M.P. Lamb. Department of Biology, Wilfrid Laurier University, Waterloo, ON.

Quantitative and qualitative evaluation of protein changes in response to biological perturbations including response to toxicants can be readily evaluated using proteomic analysis. Unlike genomics, observation of changes at the protein level allows for functional analysis of cells and organisms, and the elucidation of the toxicant's mechanism(s) of action. However, technological limitations and the complexity and variability of organismal responses make it difficult to evaluate whole organism proteomes. *In vitro* assays have been instrumental in all toxicological areas including environmental toxicology, and fish cell lines are proving crucial in screening and determining mechanisms of action of various aquatic pollutants. In mammals, comparison of cellular proteomes using cell lines has facilitated evaluation of changes due to disease or toxicant action. Thus a proteomic approach to evaluate changes in fish cell lines in response to environmental contaminants has been initiated. Protein fingerprint databases under control and toxicant exposure have been generated using 2D gel electrophoresis and computerized image analysis. Comparison of protein profiles whose expression is modulated by toxicant exposure could provide insights into the mechanisms of aquatic toxicant action and help develop new biomarkers. The expected significance of this work is far reaching and could have an economic impact in the advancement of the aquaculture and fisheries industry. (Supported by NSERC, CWN and CFI).

Identification of Phosphoproteome Components Important in Thyroid Hormone-induced Metamorphosis of the *Xenopus laevis* Tadpole. D. Domanski and C.C. Helbing. Department of Biochemistry and Microbiology, University of Victoria, Victoria, BC.

Thyroid hormone (TH) is capable of eliciting a variety of cellular outcomes including apoptosis of the frog tadpole tail and development of the brain. Central to the mechanism of TH action is a change in the transcriptome. However, there is evidence to suggest that TH directly modulates the proteome through non-nuclear receptor mediated pathways. How the proteome is involved is poorly understood and we have used the TH-induced apoptosis of the tadpole tail as well as the TH-induced changes in the tadpole brain as a model to examine this question. Signal transduction and cell cycle regulation involves changes in the phosphorylation state of proteins, therefore our approach is concerned with looking at changes in the phosphoproteome.

We have analyzed tail fin and brain proteins from control and TH-treated tadpoles by two-dimensional gel electrophoresis. Changes in protein phosphorylation were identified by western blot analyses of these gels with antibodies specific for phosphorylated tyrosine and threonine residues. Currently we have identified, by MALDI-TOF mass spectrometry, a few proteins, which undergo changes in abundance levels as a result of TH-induced metamorphosis. The procedure could have applications in identifying the protein targets of endocrine disrupting compounds.

Ecological Fate Modelling

Session Co-chairs: F.A.P.C. Gobas and B. Hickie

The Development of "Generic" and "Site-Specific" Quantitative-Structure Activity Relationship Models for Assessing Bioaccumulation Factors. J.A. Arnot and F.A.P.C. Gobas. School of Resource and Environmental Management, Simon Fraser University, Burnaby, BC.

Several countries and international organizations are evaluating chemical substances for their potential to be Persistent (P), Bioaccumulative (B) and Toxic (T) in the environment. Under the *Canadian Environmental Protection Act* (CEPA) the federal government is mandated to evaluate the P, B and T potential of approximately 23,000 substances catalogued on the Domestic Substances List (DSL). Current policies establish the bioaccumulation potential of a substance based on measured Bioaccumulation factors (BAFs), Bioconcentration factors (BCFs) and *n*-octanol/water partition coefficients (Kow). The lack of empirical data for most DSL chemicals renders quantitative-structure activity relationships (QSARs) and bioaccumulation models as necessary tools in assessing bioaccumulation potential.

In this paper, a generic QSAR-BAF model is compared to a large empirical database (2,400 BAFs/BCFs). In order to address the paucity of available empirical data this model introduces a novel approach to assess metabolic transformation and trophic dilution. Additionally, a QSAR-BAF model for deriving BAFs in a site-specific manner for the purpose of conducting screening level risk assessments is presented. The performance of this model is validated against four different empirical datasets from three fresh water systems and includes an uncertainty analysis. In summary, the development and validation of: [1] a generic BAF-QSAR model for assessing the BAF for a large number of organic substances, and [2] a BAF-QSAR for assessing site-specific BAFs in aquatic systems, are presented.

Application of Thin-Film Solid Phase Extraction to Measure Fugacity and Fugacity Capacity of Organic Contaminants in Sediments. S.V. Otton and F.A.P.C. Gobas. Department of Resource and Environmental Management, Simon Fraser University, Burnaby, BC.

Solid-phase extraction using thin films of ethylene vinyl acetate (EVA) coated onto glass provides an indirect way to measure the fugacity (or differences in fugacity) of semi-volatile organic chemicals in environmental media. Here, we examined the feasibility of using thin films to measure the fugacities and fugacity capacities of organic chemicals in marine sediments. The method is based on establishing chemical equilibria between sediments and thin films coated on the interior surface of capped 2 ml glass autosampler vials. At equilibrium, the fugacity in the sediment (fs) and EVA (fe) are equal; fe is equivalent to the ratio of chemical concentration measured in EVA to (Keva-air/RT). We will show that film-air partition coefficients of a range of chlorobenzenes correlate well with the octanol-air partition coefficients. We will further show that chlorobenzene contaminated sediments achieve chemical equilibria with the films in less than 20 minutes with insignificant losses from the sediment matrix and with coefficients of variation ranging between 15 to 20%. Finally we will demonstrate the application of the method for sediment fugacity and fugacity capacity measurements and discuss the relationship between measured fugacities and absorbable concentrations in benthic biota. We propose that the method provides a simple, quick and effective means to measure bioavailable chemical concentrations in sediments.

A Physiologically Based Toxicokinetic Model of Benzo[*a*]pyrene in the Dungeness Crab. C.V. Eickhoff[1,2], T.E. Achembach[1] and F.C.P. Law[1]. [1]Department of Biological Sciences, Simon Fraser University, Burnaby, BC; and [2]BC Research Inc., Vancouver, BC.

We have developed a physiologically based toxicokinetic (PBTK) model to predict tissue distribution of Benzo[*a*]pyrene (BP) in dungeness crab following exposure via water or food. The crab PBTK model was adapted from a previously reported rainbow trout model for pyrene but the compartmental design of the model was modified to suit the anatomy, biochemistry and physiology of the crab. The PBTK model of BP was developed based on the empirical tissue concentrations of three separate experiments in which the crabs were exposed to BP either as a bolus intravasucular injection (2 mg/kg), a single oral dose (2 mg/kg) or via the water (20 µg/L). Unchanged BP in the crab tissues of these experiments were determined by HPLC-fluorescence. The PBTK model was validated by two additional experiments in which the crabs were exposed to a 20 µg/kg single oral dose of BP or 2 µg/L of BP in seawater. Model-simulated BP tissue concentrations were compared with those of the empirical studies. BP concentrations in the haemolymph, bladder, gut, and carcass were closely predicted while those in the hepatopancreas and muscle were somewhat overpredicted. Overall the model-predicted and empirical BP tissue concentrations were in good agreement; the predicted BP concentrations are within one standard deviation of the observed values. The development of a PBTK model of BP for dungeness crab may help characterizing the risk to crabs following BP exposure or to humans consuming BP-contaminated crabs.

A Physiologically Based Toxicokinetic Model of Pyrene and 1-Hydroxypyrene in Starry Flounder (*Platichthys stellatus*). R. Namdari, T.E. Achembach, D. Sit and F.C.P. Law. Department of Biological Sciences, Simon Fraser University, Burnaby, BC.

A Physiologically Based Toxicokinetic (PBTK) model of pyrene and its metabolite,1-hydroxypyrene (1-OHP) was developed in starry flounder (*Platichthys stellatus*) after exposure to waterborne pyrene. The PBTK model consisted of eight tissue compartments: liver, kidney, gills, blood, gut, muscle, skin and carcass. Each of these compartments was described by a mass balance equation. The complete set of differential and algebraic equations was translated into Visual Basic and solved numerically with the aid of a spreadsheet program, Excel®. Absorption of waterborne pyrene by flounder was assumed to take place via the gills and the skin of the flounder. Pyrene metabolism was assumed to occur in the liver and the rate of metabolism could be described by the Michaelis-Menten kinetics. In a separate experiment, flounder were exposed to 0.8 mg/L or 8 mg/L waterborne pyrene and sacrificed at pre-selected time points. Tissue pyrene and 1-OHP concentrations were determined with HPLC. Model calibration and validation were conducted with tissue concentration data of the 0.8 mg/L and 8 mg/L experiments, respectively. Model-predicted tissue pyrene and 1-OHP concentrations described the empirical data closely. The PBTK model is a useful tool for predicting the internal doses of pyrene and 1-OHP in an ecological risk assessment of flounder after pyrene exposure (Supported by the Canadian Network of Toxicology Centres).

Modelling Sediment-Water Exchange of Organic Pollutants in Aquatic Ecosystems. F.A.P.C. Gobas and J. Armitage. School of Resource and Environmental Management, Simon Fraser University, Burnaby, BC.

Sediment-water exchange of pollutants plays a crucial role in controlling the transfer of organic pollutants in aquatic food-webs. Modelling this process is of interest is of interest in risk assessment, sediment remediation, assessment of Total Maximum Daily Loadings, source appointment and modeling the environmental fate and food-chain bioaccumulation of pollutants. Current descriptions of the sediment-water exchange of organic chemicals include equilibrium based partitioning in organic carbon, and soot carbon, particle-interaction models, as well as compartmental kinetic models accounting for deposition and resuspension. In this presentation we will show data from lake and estuarine systems that show that these models poorly estimate sediment-water distribution coefficients of organic pollutants. We will propose a mechanism based on organic carbon break-down during the sediment diagenesis that can explain the observations. We will present a novel modeling approach to estimate the sediment-water exchange of organic chemicals in aquatic systems. We will illustrate the application of the model to various systems and discuss the merits and limitations of the model.

Effects of Organic Compounds on the Marine Biota and the Role of Biotransformation: An Example with Energetic Materials. M. Nipper[1], R.S. Carr[2], J.M. Biedenbach[2], R.L. Hooten[2], B.T. Johnson[3] and K. Miller[4]. [1]Texas A&M University-Corpus Christi, Corpus Christi, TX; [2]U.S. Geological Survey, Corpus Christi, TX; [3]U.S. Geological Survey, Columbia, MO; and [4]Navy Facilities Engineering Service Center, Port Hueneme, CA.

The toxic effects of numerous organic chemicals in marine environments are well known, but the effects of their biotransformation products are mostly overlooked. It was observed in a previous survey with ordnance compounds that their biotransformation products were more toxic than the parent compounds (Nipper et al., ATW, 2000). More detailed studies on the biotransformation and toxicity of transformation products of two munitions and explosives of concern (MECs), trinitrophenol (picric acid) and 2,6-dinitrotoluene (2,6-DNT), were performed. Spiked marine sediments were used for this purpose. Biotransformation rates varied with the kind of sediment into which the ordnance compounds were spiked (sandy vs. muddy, low vs. high total organic carbon), initial total heterotrophic bacteria community, and experimental temperature. Complete biodegradation of both chemicals occurred between 3 days and 12 weeks, depending on experimental conditions. Prior to complete degradation, several biotransformation products were observed in HPLC chromatograms. The major biotransformation product of 2,6-DNT, 2-methyl-3-nitroaniline, was identified, and the identity of transformation products of picric acid are currently under analysis by GC-MS. Further toxicity assessments with the biotransformation products are underway with marine macro-algae, benthic copepods and meiofaunal polychaetes. The implications of these findings on the derivation of sediment quality guidelines will be discussed.

Selecting Bioaccumulation Criteria for Environmental Emergency Planning in Canada. L.M. White[1], K. Ketcheson[2] and M. Constable[3]. [1]Environment Canada, Dartmouth, NS. [2]Environment Canada, Ottawa, ON; and [3]Environment Canada, Edmonton, AB.

In the proposed Environmental Emergency Regulations under s. 200 of the *Canadian Environmental Protection Act*, selected chemicals are required to have environmental emergency plans provided a threshold quantity is exceeded. Chemicals were chosen based upon human health and safety criteria. In future, new chemicals added to Schedule I of s. 200 will also be evaluated using environmental (i.e., persistence, bioaccumulation and toxicity) criteria. The Bioaccumulation (B) criterion for s. 200 was developed by assessing precedence of existing criteria and comparing them by analyzing a large suite of empirical data. B criteria use threshold values for Bioconcentration Factor (BCF) and/or as a surrogate, log Kow (*n*-octanol/water partition coefficient). Data on the measured BCF and log Kow of approximately 500 nonionic chemicals were obtained from the Syracuse Research Center. Criteria were assessed by categorizing the chemicals using existing criteria and examining the relative number of chemicals exceeding the criteria, their performance relative to each parameter's distribution, and the probability of Type I and II errors. Criteria which specified both BCF and log Kow thresholds were preferred. The ultimate decision in selecting any criteria is based on a combination of science and policy. Policy issues include administrative reporting burdens and harmonization, while science issues include variability and availability in measured values, and limitations in estimating the parameters (such as the log BCF-log Kow relationship).

The Bioaccumulation of Polychlorinated Biphenyls in a False Creek Food Web Comprised of 18 Organisms. J. Maldonado[1], F.A.P.C. Gobas[2], and C.M. Mackintosh[2]. [1]Department of Biological Sciences, Simon Fraser University, Burnaby, BC; and [2]School of Resource and Environmental Management, Simon Fraser University, Burnaby, BC.

Eighteen marine organisms, sediment, and water were sampled from three sites within False Creek harbour in Vancouver, BC. Samples were sent out for PCB and stable nitrogen isotope analyses. PCB concentrations were compared to trophic position as defined by a food-chain model and by stable nitrogen isotope analysis. Results showed significantly increased PCB concentrations with increased trophic position as defined by both previously mentioned methods.

Water Borne Uptake and Tissue Distribution of Radiolabelled Glyphosate, bisphenol-A, Methoxychlor and Atrazine in Goldfish. M. Kohli, C. Rouleau and N.F. Neumann. Environment Canada, National Water Research Institute, Burlington, ON.

Pesticides such as atrazine, methoxychlor and glyphosate have been used in Ontario in quantities greater than 25,000 kg/year for crop protection. Recent evidence suggests that these pesticides may be potential endocrine disruptors. An in-depth review of the environmental toxicity and environmental concentrations of these pesticides is being examined by Environment Canada. In addition to their endocrine disrupting potential, some of these chemicals have been shown to have potent immunomodulatory effects in mammals and fish. The assessment of the environmental risks these chemicals may pose for the environment represents a formidable task.

Identification of target organs is a key data needed to orientate further research on newly investigated organic xenobiotics. In the wake of a research program evaluating the fate and effects of these chemicals in the aquatic environment, whole-body autoradiography was used to visualize the distribution of ^{14}C labelled glyphosate (Gly), methoxychlor (Met), atrazine (Atr) and bisphenol-A (BisA) in goldfish and identify target organs. Fish were exposed for 2 days (Gly and BisA) and 7 days (Met and Atr) to the radiolabelled compounds at a concentration of 5 µg/L. After exposure the fish were frozen, embedded in carboxymethylcellulose gel, and 20-µm thick cryosections were collected. Sections were freeze-dried and exposed to phosphor screens and X-ray film.

Preliminary analysis of our results show that goldfish did not accumulate Gly. The three other compounds accumulated, most of them being found in the gall bladder (ca. 80% of the body burden). Nevertheless, BisA, Met, and Atr also accumulated in other tissues, but the distribution pattern was very different. High levels of BisA were found in the gills, gill arches and specific areas of the skin. High levels of radioactivity were found in the cerebrospinal fluid of goldfish exposed to methoxychlor, whereas atrazine was quite evenly distributed in all tissues. Current studies in our laboratory has shown that atrazine and bisphenol-A are potent modulators of immune function in gold fish. A detailed analysis of the tissue distribution of these chemicals in goldfish and possibly identification of metabolites will be presented.

Dietary Uptake, Internal Distribution and Metabolism of Phthalate Esters in Staghorn Sculpin (*Leptocottus armatus*). G.M. Webster[1], F.A.P.C. Gobas[1], N. Hoover[2], C.M. Mackintosh[1] and M.G. Ikonomou[2]. [1]School of Resource and Environmental Management, Simon Fraser University, Burnaby, BC; and [2]Department of Fisheries and Oceans, Institute of Ocean Sciences, Sidney, BC.

Phthalate esters are a group of organic chemicals used mainly as plasticizers to increase the flexibility and durability of plastics. Although several phthalate ester congeners have log Kows >5, phthalates do not appear to biomagnify in aquatic food chains (Mackintosh et al. 2002). Two mechanisms may explain this lack of biomagnification: [1] dietary absorption of parent phthalate di-esters followed by metabolism to monoesters and other metabolites in the liver, or [2] metabolism (hydrolysis) of phthalate di-esters to monoesters in the gastro-intestinal tract, before di-esters can be absorbed.

To test these hypotheses, a dietary bioaccumulation experiment was conducted to measure the uptake and metabolism of phthalate esters in Staghorn sculpin (*Leptocottus armatus*). Phthalate esters were administered via the diet, and concentrations of parent di-esters and monoester metabolites were measured in the stomach contents, intestinal contents, liver tissue and muscle tissue over time. Results show the presence of monoesters in the gastro-intestinal tract, liver and muscle tissue, and a decline in phthalate di-ester concentrations from food to gastro-intestinal contents to liver to muscle tissue. A pharmacokinetic model was developed, parameterized and calibrated to quantify the uptake, internal distribution and metabolism of phthalate esters in fish. Model results are discussed in regard to the two hypotheses cited above.

Ecological Risk Assessment

Session Co-chairs: M. Macfarlane and G.P. Thomas

Overlapping Spatial and Temporal Scales in Modeling Landscape Risk. W.G. Landis, E.H. Hayes and A.M. Markiewicz. Institute of Environmental Toxicology, Huxley College of the Environment, Western Washington University, Bellingham, WA.

One of the classic questions in performing regional risk assessments is that of the appropriate of spatial scale. In fact, multiple temporal and spatial scales must be considered depending upon the management goals and assessment endpoints. We use three species within Cherry Point, a marine coastal area in northwest Washington, as a case study. We use three species, the Pacific herring, the blue heron and the Dungeness crab to contrast three very different scales. The Cherry Point run of Pacific Herring is only a few weeks in length before the fish move off shore. In contrast, the blue heron breeds locally, spends most of its lifespan within or close to the study area, and utilizes a variety of aquatic habitats. The population could be described as an isolated patch with relatively little out or in migration. Finally, the Dungeness crab comes to the region as a juvenile or larvae, and lives in the near shore region. As the crab matures it moves to deeper water, out of the study area. The crabs at Cherry Point can be considered as part of a much larger Puget Sound-Georgia Straits population. In order to understand risk assessment of this region each of these spatial scales, temporal dynamics and population structures needs to be appropriately considered.

Use of Riffle Invertebrates to Assess Water Quality in Urban Streams in the Lower Mainland, British Columbia. H.C. Bailey[1] and A. Lewis[2]. [1]EVS Environment Consultants, North Vancouver, BC; and [2]Greater Vancouver Regional District, Burnaby, BC.

Invertebrate communities have long been used as indicators of stream health. This study describes the application of this approach to urban streams in the Lower Mainland, British Columbia. The approach was validated across a range of streams, both high and low gradients, and with different levels of impact, as described by percent impervious surface in each watershed. In addition, a number of invertebrate community metrics were compared to evaluate their performance in terms of stream ranking and cost effectiveness. Invertebrate community indices were found to have a greater correlation with percent impervious surface than habitat measures. Moreover, the results suggested that similar stream rankings could be achieved using different levels of taxonomic identification.

The Relationship Between Contaminant Levels in Blood Plasma and Whole Fish Samples of Lake Trout from Several Freshwater Systems. D.M. Whittle[1], S. Newson[2], S. Brooks[3], A.T. Fisk[3], M.J. Keir[1] and R. Lazar[4]. [1]Department of Fisheries and Oceans, Bayfield Institute, Burlington ON; [2]Department of Environmental Biology, University of Guelph, Guelph, ON; [3]Environment Canada, National Water Research Institute, Burlington ON; and [4]Great Lakes Institute Environmental Research, University of Windsor, Windsor ON.

The traditional method of assessing environmental contaminant conditions in aquatic ecosystems is via the measurement of fish muscle tissue or whole body concentrations of persistent toxic substances. There are alternative fish tissues suitable as indicators of current environmental conditions. A suite of PCBs and persistent organochlorine pesticides was measured in blood plasma and whole fish samples of lake trout collected from Lake Opeongo, in Algonquin Provincial Park, Ontario, sites on Lake Superior and Lake Ontario plus Lake Champlain in upper New York State. Contaminant levels in both sample types were consistently greatest in Lake Ontario samples and lowest in samples from Lake Opeongo. Relative concentrations of all contaminants measured in both sample types were consistent over the range of fish communities surveyed. PCB was the predominant contaminant measured in all samples at all sites. Mirex and photo-mirex were measured in blood plasma samples from Lake Superior fish but were not detected in whole fish samples from the same site. Other contaminants of interest measured in blood plasma samples included halogenated phenolic compounds (HPCs). On a wet weight basis, concentrations of total PCBs in blood plasma samples were as much as 65% of those detected in whole fish tissue homogeates.

Teleost Embryotoxicity Testing of Alkyl-Phenanthrenes with a Partition Controlled Delivery Exposure Protocol. Y. Kiparissis, P. Akhtar, A. Alexander, D. Turcotte, P.V. Hodson and R.S. Brown. School of Environmental Studies, Queen's University, Kingston, ON.

There are considerable uncertainties in risk assessments and water quality criteria for hydrophobic compounds when based on conventional static exposure toxicity tests. Due to adsorption and volatization, estimated toxic concentrations often exceed solubility limits because excess chemical must be added to achieve an adequate exposure. To overcome these uncertainties, we developed a reliable, partition controlled delivery (PCD) protocol to deliver and maintain constant concentrations of hydrophobic compounds in test solutions at or below their solubility limits over extended periods. Polydimethylsiloxane (PDMS) films containing various concentrations of C1 to C4 phenanthrenes were deposited on the side of 20 mL vials and equilibrated with 10 mL of test media for 24 hours. Fertilized Japanese medaka (*Oryzias latipes*) eggs were added for a 17 day embryotoxicity test at 25°C. Results from parallel 24 hour static renewal assays demonstrated that the prevalence of blue-sac disease (BSD) symptoms in larvae increased in an exposure-dependent manner in both test regimes. In the static-renewal assays, only one compound caused toxicity below its water solubility limit, and EC50s of the other three compounds exceeded their solubility limits by 5 to 50 times. In contrast, all EC50s in the PCD assay were within the water solubility limits, indicating that this exposure protocol is a more realistic approach to assess the embryotoxicity of non-polar compounds.

Measurement of Single DNA Strand Breaks and DNA Adducts in Dab (*Limanda limanda*) for the Assessment of *in situ* Exposure to Genotoxic Compounds. F. Akcha[1], G. Leday[1], A. Pfhol-Leszkowicz[2] and Y. Cherel[3]. [1]IFREMER, Département des Polluants Chimiques, Nantes, France; [2]ENSAT, Laboratoire de Toxicologie et de Sécurité Alimentaire, Toulouse, France; and [3]Ecole Nationale Vétérinaire, Laboratoire d'Histopathologie, Nantes, France.

DNA adducts and DNA strand breaks are mutagenic lesions that have been proposed as biomarkers of genotoxicity for environmental biomonitoring. Mutations induced by these lesions in oncogenes and tumor suppressor genes are in fact believed to induce chemical carcinogenesis in vertebrates. Moreover, these mutagenic DNA lesions may have a role in the evolutionary genetics of pollutant-exposed populations. In the aim to study the relationship between marine contamination and genotoxic effects, a two-year study has been carried out in dab (*Limanda limanda*) collected from different sites of the eastern English Channel (France). DNA adducts in the liver and DNA strand breaks in blood cells were respectively measured by the nuclease P1-enhanced postlabelling technique and by an alkaline version of the Comet assay. Histopathological examinations were also realised to check for the presence of liver lesions. The results obtained during this *in situ* study will be discussed having regard to the chemical data available for the geographical area studied. For each biomarker, the results of methodological studies respectively on genotoxic identification and technical (sampling strategy) and statistical (data acquisition and data mining) aspects will be also presented. This work was supported by The Agence de l'Eau Seine-Normandie.

Dealing with the Data – Using Biotic Indices as Environmental Indicators. G.P. Thomas and K.A. Munro. G3 Consulting Ltd., Burnaby, BC.

Environmental effects monitoring for specific industries and other environmental studies include biological sampling and taxonomic work. Algae, invertebrates and fish integrate and reflect effects of the physical and chemical environment. Much is known about tolerance to specific contaminants. What do we do with all the expensive species distribution data collected? Unless we are specialists, we may feel overwhelmed by the mass of data, while suspecting there is a wealth of information to be mined. This presentation discusses strengths and limitations to use of indices. Many indices have been developed, from the Shannon-Weiner diversity index to Karr's Index of Biotic Integrity. A workable index should be based in ecological reality, be multimetric with many links to underlying processes, and integrate the data without oversimplifying. It should be descriptive, prescriptive and adaptive. Biotic indices provide tools for translating data into information, then communicating, particularly to decision makers and the public. They provide a "value-added" service, using data already collected and, occasionally, additional analyses, such as fitness indicators and presence of deformities. The approach helps provide the "so what?" of monitoring and assessment. Results can be combined with various statistical approaches and incorporated into weight-of-evidence analysis and cumulative impact, river health and risk assessments.

Defining Management Goals for a Large-Scale Ecological Risk Assessment. G. Brown[1], R.N. Hull[1], S.M. Swanson[2] and W.F. Duncan[3]. [1]Cantox Environmental Inc., Calgary, AB; [2]Golder Associates, Calgary, AB; and [3]Teck Cominco Metals, Trail, BC.

An ecological risk assessment (ERA) is being conducted for the off-site area of the Teck Cominco smelter in Trail, BC. One of the first tasks that must be completed is the identification of Management Goals for the ERA. Management Goals are statements about the desired condition of ecological values of concern. The establishment of Management Goals for the first regional-scale ERA under the B.C. Contaminated Sites Regulation is a challenging exercise, which involves all stakeholders, including the public, government and industry.

In the case of the Teck Cominco ERA, Management Goals are being developed through communication with a Technical Advisory Group (which included various government agencies), a Public Advisory Group (with interested people from the local area), and Teck Cominco (and their consultants). These Management Goals should establish clear direction for the ERA, and allow logical identification of Assessment Endpoints and Measurement Endpoints. The Management Goals, Assessment Endpoints and Measurement Endpoints for the ERA will be presented, along with a description of the process behind their development. The ERA will utilize a Weight-of-Evidence approach to characterize risks to both aquatic and terrestrial species. It will take approximately two more years to complete.

Quantitative Weight of Evidence Approach in Estimating Causal Linkages in Aquatic Systems. W.G. Landis. Institute of Environmental Toxicology, Huxley College of the Environment, Western Washington University, Bellingham, WA.

A classic problem in aquatic toxicology is the estimation of the causes of impacts observed in aquatic populations and communities. I used a weight of evidence (WoE) approach based upon our relative risk model in order to estimate the cause of the decline of the Cherry Point Pacific herring since the early 1970s. This WoE approach is based upon a risk assessment type conceptual model in order to link potential sources of stressors to the effects seen in the population. Ranking criteria are used to assign weights to the potential sources and stressors. Other criteria are used to establish filters to establish linkages between the stressors and the observed effects. A Monte Carlo analysis is applied to represent the uncertainty in each of the ranks and filters and to estimate the sensitivity of the models. This technique results in a series of multinomial distributions representing the likelihood of a stressor causing an impact. In the case of the Cherry Point herring, climate change, habitat alteration and widespread contamination were identified as important stressors. The method also pointed to key uncertainties that require further laboratory and field investigation. This case study demonstrates that a clearly derived and quantified WoE is a useful approach to investigating casual links.

Weight of Evidence Determinations in Ecological Risk Assessment. P.M. Chapman. EVS Environment Consultants, North Vancouver, BC.

Weight of evidence (WOE) frameworks for integrating and interpreting multiple lines of evidence will be discussed, focusing on sediment quality assessments. Approaches to WOE include individual lines of evidence (LOE) as well as combined LOE (indices, statistical summarization, logic systems, scoring systems, and best professional judgment [BPJ]). The application of WOE, based on multiple LOE, will be discussed relative to the published literature. Fully implementing WOE requires consideration of six main LOE in sediment (or other assessments); these LOE generally correspond to other causality considerations including Koch's Postulates. However, the issue of sediment stability is an additional consideration, and the use of tabular decision matrices is recommended in a logic system to address LOE described by others as "analogy", "plausibility", or "logical and scientific sense".

Three examples of logic system WOE determinations based on the Sediment Quality Triad and using tabular decision matrices will be outlined and discussed. Key lessons from these examples include the: generally limited utility of sediment quality value (SQV)-based LOE; need for BPJ; importance of ecological relevance; importance of assessing background conditions; and, need for appropriately customizing study designs to suit site-specific circumstances (rather than application of "boiler-plate" assessments). Overall, more quantitative approaches are needed that better define "certainty elements" of WOE in an open framework process, i.e., statistical summarization culminating in a logic system incorporating BPJ.

Development of a Protocol for Providing Relevant Toxicity Data for Chemical Spill Contingency Planning. L.M. White[1] and K.G. Doe[2]. [1]Environment Canada, Dartmouth, NS; and [2]Environment Canada, Moncton, NB.

In cases of accidental release of toxic chemicals, the most likely type of scenario is of >pulse= exposure where the chemical is released at a very high concentration in a relatively short time period. Our aim was to develop a protocol for use in contingency planning which would produce an economical, scientifically defensible set of short-term >pulse= toxicity data to estimate the adverse risk of chemical spills to aquatic organisms. A suite of acute lethality and subchronic static tests with four chemicals (acrolein, acrylonitrile, cadmium chloride and potassium dichromate) and two fish, one invertebrate and one plant species were used. We also took an environmentally realistic approach: organisms were exposed to chemicals for 1, 4 and 10 hours and then transferred to clean water for the normal duration of the relevant test. Similar to previous studies with other species, continuous exposure tests are poor predictors of the toxicity of short-term exposure. Most acute lethal endpoints of one and four hour exposures were significantly different from, and up to 64 times greater, than those of continuous exposure tests. Based upon species sensitivity, our results indicate that estimates of the toxicity of short-term exposures should include acute lethal tests of both an invertebrate and a fish species. Microtox tests were also conducted and were the most sensitive endpoint for one hour exposure. Further testing is required to determine the usefulness of Microtox® to protect all species.

Hazard Evaluation Framework for Environmental Emergency Plans Under Sections 199/200 off *Canadian Environmental Protection Act 1999*. M. Constable[1], K. Ketcheson[2] and L.M. White[3]. [1]Environment Canada, Environmental Protection Branch, Edmonton, AB; [2] Environment Canada, Environmental Emergencies Branch, Hull, QC; and [3]Environment Canada, Environmental Emergencies Branch, Dartmouth, NS.

This poster presents the approach being taken by Environment Canada in developing a model of how to determine when a chemical requires an environmental emergencies plan under Section 200 of *Canadian Environmental Protection Act 1999*. The approach includes parameters for human health, environmental and physical impacts during emergency releases or spills from fixed facilities. The method relies heavily on established criteria for hazardous properties of chemicals, as well as some criteria that have been developed specifically for this purpose.

The model, called the Hazard Evaluation Framework (HEF), consists of a set of criteria that incorporate the criteria used by the U.S. EPA to develop their Risk Management Program list of air toxics, as well as other criteria to select chemicals for potential environmental hazards. The chemical evaluator uses the HEF and its associated criteria to assign scores to the various parameters of the chemical in question. The scores are entered into an Excel spreadsheet that manipulates them and generates a ratio that can be used to determine if an Environmental Emergency Plan is required.

Screening Canada's Domestic Substances List: What to do First? M. Eggleton, P. Cureton and R. Chenier. Environment Canada, Existing Substances Branch, Hull, QC.

Under the *Canadian Environmental Protection Act 1999*, all substances identified as persistent and/or bioaccumulative and inherently toxic during the categorization process of Canada's Domestic

Substances List (DSL) must undergo a screening level environmental risk assessment (SLERA) to determine whether the substance may constitute a threat to the Canadian environment. Considering the high number of substances to be screened, an efficient and effective procedure must be developed for prioritizing and carrying out these assessments.

A pilot project is currently being conducted for the screening of 123 DSL substances of various classes. The OECD has already established a program for member countries to share information regarding environmental assessments of high production volume chemicals. It entails the use of Screening Information Data Sets (SIDS) for preparing Initial Assessment Reports (SIAR) about a chemical's hazards. It is in the interest of Canada to make its own screening procedures compatible with this international process.

Bioavailability and Toxicity of Contaminants to *Corophium volutator:* Field and Laboratory Experiments. J. Hellou[1], K. Cheeseman[2], E. Desnoyers[3], A. Gronlund[2], D. Johnston[3], J. Leonard[1], S. Robertson[2] and S. Steller[1]. [1]Department of Fisheries and Oceans, Bedford Institute of Oceanography, Dartmouth, NS; [2]Department of Biology, Dalhousie University, Halifax, NS; and [3]Department of Chemistry, Dalhousie University, Halifax, NS.

The assessment of risk associated with exposure to contaminants will depend on the characteristics of the species studied and our ability to follow up on effects in field or laboratory experiments. Small benthic animals such as amphipods are good to use in laboratory investigations and can help to assess the factors involved in affecting ecosystem health, even when not available in the field. The binding of hydrophobic chemicals to sediments will depend on their physical-chemical properties, perhaps their source, age, as well as the grain size and total organic carbon (TOC) of sediments under consideration. The fate of polycyclic aromatic compounds (PACs), abundant priority pollutants in coastal sediments was investigated using a marine amphipod, *Corophium volutator*, collected inter-tidally in Nova Scotia and exposed in the laboratory to Halifax Harbour sediments. The bioavailability of PACs was determined by examining their bioaccumulation in amphipods with exposure time, using different groups of animals and post-depuration. Biota-sediment accumulation factors (BSAF) were compared for a suite of compounds and for sediments from various locations in the harbour. Biological effects, i.e. amphipods survival was not affected and females reproduced at all sites. Lipid content and growth were also examined in the short-term and interpreted relative to chemical and biological results obtained on inter-tidal mussels collected in the field. The limitations and advantages of a controlled laboratory set up will be discussed relative to field conditions faced by mussels over years of exposure.

Use of *Canadian Environmental Quality Guidelines* in the Risk Management of "CEPA-Toxic" Nonylphenol and its Ethoxylates. K.L. Potter[1], D.J. Spry[1], J.-F. Ferry[2] and F. Huppé[2]. [1]Environment Canada, National Guidelines and Standards Office, Hull, QC; and [2]Environment Canada, Toxic Substances Environmental Protection Branch, Montreal, QC.

In a 1999 report, the Commissioner on the Environment and Sustainable Development (CESD) identified the need for clearly defined environmental objectives and the use of environmental performance measures in the risk management of substances found toxic under the *Canadian Environmental Protection Act* (CEPA). One of the steps taken towards addressing the CESD's concerns was a pilot project to examine how Canadian Environmental Quality Guidelines for the CEPA-toxic nonylphenol and its ethoxylates (NPEs) could be used in developing a risk management strategy. Canadian Environmental Quality Guidelines (CEQGs) can play a role in setting environmental objectives, determining risk management objectives, selecting management instruments, and evaluating the effect of those management actions on environmental quality. Examples from the case study with NPEs are provided to illustrate some of the options available for using CEQGs at each stage of the risk management process. Risk management objectives for NPEs were developed with the purpose of achieving the environmental objectives of 1 μg/L of nonylphenol toxic equivalency units (NP TEQ) in fresh waters and 0.7 μg/L NP TEQ in marine waters.

To Model or Measure - The Risk Assessment Dilemma. G.A. Wickstrom and M.A. Cameron. Keystone Environmental Ltd., Burnaby, BC.

One of the first decisions to be made during the planning stages of an ecological risk assessment is to determine what exposures to model and what to measure. Direct measurements of environmental media (e.g., soil, sediment, surface water) is common practice, but when it comes to determining exposures to ecological receptors, many elect to model using assumptions and available literature information. There are many pros and cons associated with both modeling and measuring exposure concentrations in potential ecological receptors. The information presented herein is not intended to be an exhaustive investigation into right versus wrong, but rather a presentation of some of the major pros and cons of each approach and their implications on the ecological risk assessment process. In general, measuring ecological exposures provides results with greater accuracy, assuming appropriate study design, but may not be practical or possible under all circumstances. Modeling may be more practical and more easily standardized, but is limited by the constraints of the model, the assumptions made, and generally produces results with greater uncertainty. The advantages and disadvantages of these exposure assessment methods are numerous and the decision of which approach to use should be made within the context of the overall study objectives and realities.

A Proposed Framework for Determining Persistence, Bioaccumulation and Ecotoxicity of Organic Substances: Mandate under the *Canadian Environmental Protection Act* 1999. J.C. Sanderson, M.L. Lewis, N. Davidson, P.D. Robinson, M.J. Shaw and L. Boulanger-Stewart. Environment Canada, Existing Substances Branch, Gatineau, QC.

Under Sections 73 and 74 of the revised *Canadian Environmental Protection Act (CEPA 1999)*, Environment Canada is mandated to "categorize" and then, if necessary, "screen" the approximate 23,000 substances on Canada's Domestic Substances List (DSL) for persistence (P), bioaccumulation (B) and inherent toxicity (iT) to non-human organisms. Nearly one half of the DSL is comprised of discrete organic chemicals. The approaches for determining persistence, bioaccumulation and inherent toxicity to non-human organisms have been examined and a framework has been proposed by which organic substances will be categorized.

The proposed guidance describes how pivotal values are selected from experimental data and QSAR predictions as well as how these are subsequently measured against the P, B, and iT criteria established by Environment Canada. The P and B categorization criteria are defined in the *Persistence and Bioaccumulation Regulations* and the iT criteria are currently under development. As there is a paucity of experimental data for many of the organic substances on the DSL, particular attention will be given in this presentation to the proper selection and use of QSARs, as advocated by the QSAR workshop hosted by Environment Canada in 1999. This presentation will summarize the rationale and framework of the proposed categorization approach for discrete organic chemicals.

Does Biomagnification Have a Role in Hazard Assessment of Metals? S. Schnabel, P. Doyle, Y. Couillard, D.W. Gutzman. Environment Canada, Existing Substances Branch, Hull, QC.

The *Canadian Environmental Protection Act* (CEPA 99) requires that the persistence (P), bioaccumulation (B), and inherent toxicity (iT) of all commercial chemicals listed on the Domestic Substances List (DSL) be evaluated by September 2006. Substances found to be P and/or B and iT, will undergo a screening level risk assessment. A substance is defined as bioaccumulative if its bioaccumulation or bioconcentration factor is equal to or greater than 5000, or for organic compounds if its log Kow is equal to or greater than 5. Organic substances with these characteristics typically have the potential to biomagnify. For completeness and consistency, it is desirable to apply the PBiT approach to organic and inorganic substances in a similar manner. It is recognized that for some metals active uptake processes make interpretation of bioaccumulation and biomagnification very difficult. However, there is growing empirical evidence that metals can have the potential to biomagnify, some directly (e.g., Cd) and others indirectly via biomethylation (e.g., Hg, Se), with potentially negative environmental consequences. There is a need for further research to assess processes of methylation and demethylation in the environment and mechanisms of metal uptake and depuration in organisms. One approach is the further development of models for bioaccumulation and biomagnification of metals in biota. Close cooperation between researches in universities, government, and industry is required if we are to succeed in accurately interpreting information on biomagnification and incorporating this into hazard assessments for metals.

Characterization of the Quaternary Ammonium Component Found in Alkaline Ammoniacal Copper Quaternary Wood Preservative (ACQ). A. Moore. Environment Canada, Environmental Protection Branch, Edmonton, AB.

Alkaline copper quaternary compounds (ACQ) are being proposed as alternatives to the current wood preservative CCA, which contains the potentially hazardous substances arsenic and chromium. ACQ contains two different fungicidal ingredients; copper is the primary one and a quaternary ammonium compound (quat) is the secondary one. The quat component of ACQ is either alkyldimethylbenzylammonium chloride (ADBAC) or didecyldimethylammonium chloride (DDAC). Both of these compounds already have widespread use in Canada as disinfectants, sanitizers, antisapstains, biocides and preservatives. These compounds are soluble in water, nonvolatile, non-bioaccumulative and they sorb readily to sediments. Degradation rates are highly variable from days to years depending on the area-specific conditions. Both compounds are highly toxic to invertebrates and fish with the majority of LD50s being under 1.0 mg/L. This project characterizes these general properties of the quat components found in ACQ as well as their potential entry, exposure and effect on the Canadian environment from their use in other products.

Toxicological Significance of Photomodification of Polycyclic Aromatic Hydrocarbons in the Environment. X.-D. Huang, J. Nykamp, M. Lampi, S.T. Babu, Y. El-Alawi, J. Carlson, N.C. Bols, D.G. Dixon and B.M. Greenberg. Department of Biology, University of Waterloo, Waterloo, ON.

Polycyclic aromatic hydrocarbons (PAHs) are found in most industrial environments. They are priority contaminants because of their acute toxicity, mutagenicity and carcinogenicity. Sunlight exposure of PAHs increases their toxicity and carcinogenicity. Two mechanisms are involved in photoinduced toxicity of PAHs, photosensitization and photomodification. Through photosensitization, PAHs generate reactive oxygen species. By photomodification process, PAHs generate photomodified products, usually oxyPAHs. These oxyPAHs have shown to be more toxic and carcinogenic than the parent compounds. In particular, if the oxyPAHs are present in a mixture, the toxicity is greatly enhanced.

In the past several years we have investigated presence and toxicity of these oxyPAHs in aquatic environments and found that oxyPAHs are widely present in the environment. In many cases, the concentrations of oxyPAHs are comparable with that of PAHs. More importantly, mechanisms of toxicity of these oxyPAHs are different from PAHs. They are concentrated in mitochondria or chloroplast and interfere with electron transport reactions in mitochondria or chloroplast. Some of them are enzyme inhibitors, while others intercept electrons from electron transport reactions and pass on to oxygen generating reactive oxygen species. Synergetic effects of mixtures of PAHs and oxyPAHs are often observed in toxicity testing. This may partially explain why toxicity and/or risks of PAH mixtures in the environment are often underestimated.

Ecotoxicological Potential of Polycyclic Aromatic Hydrocarbons for Fish: Need for a Broader Risk Assessment. A. Mathieu[1], J.F. Payne[2] and T.K. Collier[3]. [1]OCEANS Ltd., St. John's, NF; [2]Department of Fisheries and Oceans, St. John's, NF; and [3]Northwest Fisheries Science Center, Seattle, WA.

The toxicology of PAH dates back to observations made in the 1700s on scrotal cancers in young chimney sweeps in London and in the 1800s on skin cancers in coal tar workers in Germany. Environmental concerns have also arisen in more recent times about elevated levels of PAH entering the aquatic environment from petrogenic and pyrolytic sources. However PAH still receive scant attention by management and environmental interests in many countries and jurisdictions. To what extent are these compounds of ecotoxicological importance? We have evaluated field and laboratory studies carried out over the past two decades or so linking various biological effects in fin-fish to PAH. Information on biochemical, histopathological, genetic, immunological, reproductive, developmental and behavioural effects have been reviewed and assessed according to: [1] field studies, [2] laboratory studies with complex mixtures of PAH, and [3] laboratory studies with individual PAH. Linkages have also been made in relation to levels of PAH in many waters and sediments and associated guidelines. Establishing a "legal" standard of causal evidence for environmental effects of PAH seems improbable. However, considering the combination of field and laboratory studies presently available and using a weight of evidence approach, we suggest that levels of PAH commonly found in many marine and freshwater environments are causing or contributing to health effects in fish. Our review also points to a need for a risk assessment of the effects of PAH on fish larvae, which according to reported sensitivities and reported levels of PAH in many waters, including away from non-point sources, may be at sufficient risk to impact fish recruitment.

Follow-Up Reports from the First Priority Substances List: An Update. N. Davidson, P. Cureton, W. Windle, K. Taylor and K. Mailhiot. Environment Canada, Existing Substances Branch, Gatineau, QC.

The first Priority Substances List (PSL1) was published in 1989, under the *Canadian Environmental Protection Act (CEPA)*, and included 44 substances or groups of substances. Environmental and human health risk assessments were completed by Environment Canada and Health Canada under the Priority Substances Assessment Program in early 1994. Assessment Reports for each of the PSL1 substances involved a critical review of relevant and current entry, exposure and effects data. The purpose of the assessments was to determine whether or not a substance was "toxic" as defined by *CEPA*. Under *CEPA*, a substance is considered "toxic" if it enters or may enter the environment in amounts or under conditions that may pose a risk to human health, the environment or its biological diversity, or to the environment on which life depends. However, for several of the PSL1 substances, data available at that time, were considered insufficient to conclude whether these substances were "toxic." Since then, both Environment Canada and Health Canada have addressed the data gaps and have reached proposed conclusions on the toxicity of these substances. An update on the findings and status of these PSL1 Follow-up Reports is presented below.

Preliminary Screening Level Ecological Risk Assessment of Polybrominated Diphenyl Ethers in Canada. J. Pasternak[1], K. Taylor[2], L. Suffredine[1] and L. Lander[2]. [1]Environment Canada, Environmental Protection Branch, North Vancouver, BC; and [2]Environment Canada, Existing Substances Branch, Gatineau, QC.

The *Canadian Environmental Protection Act* (CEPA 1999) requires the Ministers of the Environment and Health to "categorize" substances on the Domestic Substances List (DSL) and if found to be PiT (Persistent and Inherently Toxic), BiT (Bioaccumulative and Inherently Toxic), PBiT (Persistent, Bioaccumulative and Inherently Toxic) or PB (Persistent and Bioaccumulative) with greatest potential for human exposure, then "screen" to determine whether they are "toxic" or capable of becoming "toxic" as defined in the Act. As part of a Pilot Project study under this Categorization and Screening program, seven polybromodiphenyl ethers (PBDEs) were selected to undergo a screening level risk assessment (SLRA). PBDEs are used extensively as flame retardants, and in recent years there has been concern regarding the possibility of ecological impacts associated with their release to the environment. The seven PBDE congeners evaluated in the SLRA are present in three commercial mixtures, pentabromodiphenyl ether (PeBDE), octabromodiphenyl ether (OBDE) and decabromodiphenyl ether (DBDE).

The SLRA assessment process examined exposure and toxicity data for each commercial mixture and its major components to determine an estimated exposure value (EEV) and estimated no-effects value (ENEV) for water, sediment, soil and biotic compartments. Risk quotients were derived by dividing the EEV by the ENEV. The quotient was used to make quantitative and qualitative statements about the potential for risk due to PBDE congeners, based on conservative assumptions, in that compartment. The calculated risk quotients were below one with respect to pelagic organisms for PeBDE, OBDE and DBDE. For benthic organisms, EEVs exceeded ENEVs for PeBDE, but not for OBDE or DBDE. However, hexaBDE has demonstrated toxicity and is a component of both PeBDE and OBDE. Therefore, risk associated with PeBDE may also occur with the use of OBDE due to the presence of the hexaBDE congener. For soil organisms, the risk quotient exceeded one for DBDE, but not for the other products. The risk quotients for secondary poisoning exceeded one for PeBDE and OBDE, but not DBDE. The assessment found that tetra-, penta- and hexaBDE are highly bioaccumulative and each congener has a reported bioconcentration factor (BCF) that exceeds 5000 for aquatic species. Empirical and predicted data indicate that all PBDE congeners subject to this SLRA are also highly persistent. Tetra-, penta-, hexa- and heptaBDE have been measured in the arctic environment which indicates being subject to long range atmospheric transport. There is uncertainty regarding the environmental fate of the third commercial product, DBDE. Further information on the possible debromination products of DBDE and its potential to bioaccumulate is an important data gap. This assessment will be subject to peer review and public comments, prior to the determination of a conclusion on CEPA toxicity.

Emerging Toxicological Issues and Approaches

Session Co-chairs: G.L. Stephenson and J. Miller

Application of Phytotechnologies to Contaminated Soils. J.J. Germida. Department of Soil Science, University of Saskatchewan, Saskatoon, SK.

Phytotechnologies is the name given to a set of emerging environmental cleanup technologies that use plants to enhance the dissipation or stabilization of environmental contaminants– including petroleum hydrocarbons (PHCs), salts and metals. The advantages associated with phytotechnologies reflect the fact that plants have extensive root systems that help to bring microbes, nutrients, and contaminants into contact with each other and, thus greatly increase the volume of soil in which active microbial degradation can be stimulated. Moreover, microbial populations and activities in the rhizosphere can be 5 to 100 times greater those than in the bulk soil which, in turn, can lead to enhanced contaminant degradation in the rhizosphere. Contaminated soils pose a significant threat to human and ecosystem health in Canada. For example, the number of petroleum hydrocarbon-contaminated sites in Canada is estimated at more than 400,000 and the cost associated with their remediation is in the billions of dollars. Given that petroleum hydrocarbons are amenable to microbial degradation and that plant-based bioremediation (i.e., phytoremediation) often involves enhanced microbial degradation of contaminants in the rhizosphere, oil contaminated sites are good candidates for phytoremediation. Nevertheless, despite the flexibility and adaptability that various plant-associated remediation pathways provide, interactions between these pathways as well as the biochemical and ecological interactions between the plant-microbe-environment continuum give rise to high level of complexity surrounding phytoremediation. Understanding this complexity is crucial to the success of any phytoremediation effort. Here, I discuss the key processes involved in phytoremediation, its promises and limitations, and its applicability to the harsh environments (semi-arid, short growing season, and long, cold winters) characteristic of the oil-producing regions of western Canada. The focus of this work is on the selection of native plants for phytoremediation, the assessment of the microbial diversity associated with the roots of these plants and the development of bacterial inoculants to enhance phytoremediation of petroleum hydrocarbons.

Soil Invertebrate Toxicity Test Methods with Relevance to the Canadian Environment – Lessons Learned from Tests on Salt Toxicity. J.A. Addison and D.A. Bright. Applied Research, Royal Roads University, Victoria, BC.

British Columbia recently developed draft soil standards for salt ions to better facilitate the remediation of sites affected by road salt and produced water. The lack of pre-existing data for salt ions provided an opportunity to carry out soil invertebrate toxicity testing, with an emphasis on new and evolving methods chosen for their relevance to British Columbia field settings. For examples cultures of the following collembolans were established: *Folsomia candida, Onychiurus folsomi, Protaphorura armata, Proisotoma minuta,* and *Folsomia nivalis.* It was concluded that soil type has a profound influence on NaCl toxicity, underscoring limitations of use of an OECD soil, adjusted to neutral pH, for predicting field responses in BC, where soil pH tends to be in the pH 3.5 to 6.5 range. In addition, there was a large dependence of dose-response relationships on the degree of soil saturation at which tests were run. This may have implications for field responses during periods of drought. Sub-chronic mortality invariably occurred at much higher soil salt concentrations (by a factor of five or more in several cases) than longer-term reproductive impairment. Indeed, thresholds of acute or sub-chronic mortality of most soil invertebrates would not be expected to be a good predictor of longer-term population fitness, nor community structure, since the fauna are adapted to withstand extremes in micro-environmental conditions over short periods through avoidance-type behaviours and inactivity. Such inactivity, however, is accompanied by lack of feeding and reproduction, a necessary activity for over-wintering survival of most species.

Development of a New Environment Canada Test Method for Measuring Survival and Reproduction Effects in Soil Using Springtails (*Onychiurus folsomi* and *Folsomia candida*). J.A. Miller[1], R.P. Scroggins[2], G.L. Stephenson[3] and K. Becker-van Slooten[4]. [1]Miller Environmental Sciences Inc., Innisfil, ON; [2]Environment Canada, Method Development & Applications Section, Ottawa, ON; [3]Aquaterra Environmental, Orton, ON; and [4]CECOTOX, École Polytechnique Fédérale de Lausanne, Switzerland.

In 1994, Environment Canada (EC), the Canadian Association of Petroleum Producers (CAPP) and the Program of Energy Research and Development (PERD) initiated a multi-year program to develop biological test methods that could be used to assess the toxicity of contaminants in soils using terrestrial organisms. The goal was to develop test procedures that were applicable to Canadian soil types using terrestrial species that were representative of Canadian soil ecosystems.

The creation of a new Environment Canada test method for measuring survival and reproduction effects in contaminated soil using springtails is the final phase of the soil toxicity test method development program. The new toxicity test is based on research conducted by Aquaterra Environmental, ESG International, École Polytechnique Fédérale de Lausanne and the University of Guelph. The principal test is a definitive 28 or 35 day test for measuring chronic toxicity using survival, adult fecundity and number of live juveniles as endpoints. An acute lethality screening test will also be described in the new method. The two collembolan species recommended as test organism choices are *Onychiurus folsomi* and *Folsomia candida*. Key components of the test method and rationale for selection of test specifications will be discussed.

Sediment PAH Phototoxicity - An Ecologically Irrelevant Phenomenon? B.G. McDonald and P.M. Chapman. EVS Environmental Consultants, North Vancouver, BC.

Photo-enhanced toxicity of polycyclic aromatic hydrocarbons (PAH) is well demonstrated in laboratory and in a few *in situ* studies. Effects have been observed for multiple taxa and toxicological endpoints, and the mechanism of toxic action has been described. However, this phenomenon is ameliorated by physical, chemical and biotic factors; "the unanswered question...into the phototoxicity of contaminated sediment (and water) is whether phototoxicity is of ecological relevance or merely an interesting laboratory artifact" (Boese et al., 1999). To date there have been no studies that clearly and directly implicate PAH phototoxicity with adverse ecological effects in field populations. If phototoxic effects were present in natural environments at the same magnitude as those observed in published studies, large areas of shallow aquatic environments should be depauperate; yet this is not the case. We have worked at sites where phototoxicity was demonstrated for particular organisms, yet these same organisms remained abundant at those sites. Determination of the ecological relevance or lack thereof of PAH phototoxicity should supplant continued publication of ecologically irrelevant laboratory studies. Environmental management decisions should not, without a determination of ecological relevance, be based on laboratory-based phototoxicity studies.

Biocides for the Control of Aquatic Nuisance Species in Ballast Water. M. O'Reilly[1], K.E. Holtze[1] and D. Stocks[2]. [1]ESG International Inc., Guelph, ON; and [2]BMT-Fleet Technology Ltd., Kanata, ON.

The economic impact of aquatic nuisance species (ANS) invasions in North America is estimated in the billions of dollars and the ecological impacts are not yet fully understood. In an effort to limit introductions of ANS via the ballast water vector, numerous treatment technologies are being investigated including the used of biocides. A recent study was conducted to determine the practicality of using two biocides, hypochlorite and copper ion, to treat ballast water before discharge in port. The investigation included aquatic toxicity testing in the laboratory, preliminary field-tests onboard a bulk carrier vessel, and corrosion testing in a materials laboratory. The work investigated among other things, the effectiveness of the biocides in inactivating species of concern, water quality issues, disinfection by-products, and health and safety. The results of the study indicate that while both biocides have potential benefits for reducing ANS invasions through ballast water discharges, environmental, practical, and operational issues surrounding the use of each require further attention.

Use of Tests with Fish Cell Lines as an Alternative to Toxicity Tests with Fish. V.R. Dayeh[1], L.E.J. Lee[2] and N.C. Bols[1]. [1]Department of Biology, University of Waterloo, Waterloo ON; and [2]Department of Biology, Wilfrid Laurier University, Waterloo, ON.

Fish cell lines have long been considered as alternatives to fish in toxicity testing, but several technological advances and the increasing demand to test industrial effluents has made animal cells more attractive. Fish cell lines have been developed from most organs of bony fish and can be grown continuously and cryopreserved indefinitely. As a result of cell lines being immortal, there is no need to periodically initiate cultures from fish as is necessary with primary cultures. Except for temperature, fish cells can be grown in a manner similar to mammalian cells. They do not grow as rapidly as mammalian cells, which is a disadvantage, but appear to be more logical substitute for fish. Fish cell line tests have several advantages over fish tests: they satisfy a societal desire to reduce animal use in toxicity testing, can be done more quickly and at less cost, and provide insight into toxicity mechanisms. Recently, the effectiveness of using a fish cell line (RTgill-W1) as a screening tool was studied by examining the toxicity of thirty one whole-water samples from a paper mill. The only sample toxic to rainbow trout was the only sample cytotoxic to RTgill-W1. Therefore, fish cell lines seem to be a promising substitute for fish in toxicity testing of industrial effluent.

Modelling Multiple Metal Interactions at Fish Gills and other Biotic Ligands. R.C. Playle. Department of Biology, Wilfrid Laurier University, Waterloo, ON.

There are various developing biotic ligand models, and more specifically there are published metal-gill interaction models for Cu, Cd, Ag, Co, and Pb, and partial metal-gill models for Al and Zn. These models assume that the amount of metal binding to a sensitive biological membrane determines the metal's acute toxicity. Competition at the membrane (e.g., by Ca) and complexation in the water itself (e.g., by natural organic matter and by carbonate) reduces metal binding to the membrane and therefore reduces metal toxicity. Conceptually, these models consider the biological membrane as a ligand with a particular metal binding strength, so mathematically bridge the gap between water chemistry and metal toxicity to organisms. But how do these models handle multiple metals? Using the classic toxic unit concept, a two metal-gill interaction model predicts strict additivity at 0.5 T.U. plus 0.5 T.U. (=1.0 T.U.), predicts greater than strict additivity at 0.25 T.U. plus 0.25 T.U. (>0.5 T.U.), and predicts less than strict additivity at 0.75 T.U. plus 0.75 T.U. (<1.5 T.U.). These deviations from strict additivity are a result of fast filling of binding sites at low metal concentrations and strong competition at high metal concentrations (e.g., non-linearity of the models), with a crossover at, perhaps not coincidentally, 1/n T.U. + 1/n T.U. + 1/n T.U. ... = 1, where n is the number of metals.

Challenges in Hazard Assessment of Genetically-Modified Biotechnology Products in the Environment. M. Douville, F. Gagné and C. Blaise. Environment Canada, Centre Saint-Laurent, Montréal, QC.

In Canada, genetically-modified corn and *Bacillus thuringiensis* (Bt) application are commonly used for pest control in many types of crops. For example, corn was genetically-modified to express the Cry 1Ab endotoxin (so-called Bt-corn) which is toxic to insects from the *Lepidoptera* family. Native and Bt-corn is by far the most cultivated crop in our region. A project consisting of two main objectives was initiated to gain some knowledge for preliminary hazard assessment: [1] determine the levels of Cry1Ab endotoxin and its gene in surface waters and sediments near a Bt-corn field, and [2] determine toxic effects to non-target aquatic organisms of Cry1Ab. The life-cycle of the cry1Ab toxin in the (aquatic) environment must be understood in order to identify whether these proteins originate from Bt-corn or naturally-occurring Bt.

Preliminary results indicate that extractable Cry1Ab toxin at pH 7.4 is sometimes found in the aquatic environment at very low levels; the highest concentration found was in the order of 5 µg/kg and 0.5 µg/L in sediments and surface waters, respectively. The data also showed that levels of pH 7.4 extractable Cry 1Ab were not significantly correlated ($p>0.1$, rank correlation) with those of Bt counts suggesting independence of Cry1Ab towards levels of BT. Validation experiments are currently in progress to address the persistence of the endotoxin in different types of soils or surface waters and to determine if Cry1Ab from naturally-occurring Bt (protoxin) can be degraded in the soluble form (Bt-corn). Measurements of gene release in the environment as determined by real-time PCR analysis of extracted DNA from soils, sediments and surface waters are also planned. Some recommendations will be proposed for following genetically-modified organisms and their products in the environment. Project funded by Environmental Management of Biotechnology for Regulation and Research, Jim Louter.

Acute Toxicity of Rare Earth Metals to *Daphnia pulex*. M. King, M.L. Schwartz and J.C. McGeer. Natural Resources Canada (NRCan), Ottawa, ON.

The *Canadian Environmental Protection Act* (CEPA, 1999) requires the categorization of substances on Canada's Domestic Substances List (DSL). Categorization involves evaluation of the substances on the basis of their persistence, bioaccumulation and inherent toxicity (P, B & iT) , which is similar to hazard identification. Substances found to be P and/or B and iT, will undergo a screening level risk assessment. For many metals and inorganic substances on the DSL, important data gaps in relation to toxicity makes the process of categorization very difficult. To address this situation, NRCan in partnership with Environment Canada, has embarked on an investigative project to characterise the toxicity of 21 elements, primarily the rare earth or lanthanide metals. The objective of the NRCan portion of the study was to develop high quality acute toxicity data for the data poor metals toward daphnids. Using standard methods, we tested the acute toxicity of 21 metals to *Daphnia pulex* in very soft water (hardness of 12 mg/L as $CaCO_3$, pH of 7 to 8). The approach we used was adapted to the proposed framework for categorizing inorganic substances on the DSL, with concentrations of 1000, 315, 31.5, and/or 10 ug/L being tested for each metal. The results show the relative toxicity of these metals and will play an important role in contributing data towards categorization and possibly the screening level risk assessment process which may follow.

Developments in *Lemna minor* testing: A Comparison of International Standards Organization and Environment Canada Methods. M. Moody[1] and R.P. Scroggins[2]. [1]Saskatchewan Research Council, Saskatoon, SK; and [2]Environment Canada, Ottawa, ON.

Use of sublethal test methods taking place in many countries is leading to the development and publication of international standards by groups such as the International Standards Organization (ISO). The draft ISO standard (ISO/CD 20079) and Environment Canada test method (EPS 1/RM/37) both require the use of *Lemna minor* as test organism. Both methods outline procedures for the determination of growth inhibition when the test organism are exposed of substances such as chemicals or plant protection products, contaminant mixtures in water, treated municipal or industrial effluents. However, the testing medium (modified APHA) of the Environment Canada method has been specifically fine tuned for the testing of metal compounds and metal mining effluents. This study was undertaken to investigate aspects of the ISO methodology that might be lead to recommended changes in either the Environment Canada method or the draft ISO standard. Focused research included investigation of test media, lighting conditions, containers and use of black background. Testing of four metals (Zn, Cu, Cd and Ni) was carried out in two test media according to the ISO protocol. Recommendations include that each protocol adopt aspects of the other.

Endocrine Modulating Effects of Confined Animal Feed Operation Discharges. D.M. Janz[1], J.N. Dumont[2], L.P. Weber[2] and S.R. Hutchins[3]. [1]Department of Veterinary Biomedical Sciences and Toxicology Centre, University of Saskatchewan, Saskatoon, SK; [2]Department of Zoology, Oklahoma State University, Stillwater, OK; and [3]U.S. Environmental Protection Agency (NRMRL/SRPD), Ada, OK.

Although confined animal feed operations (CAFOs) are a potential source of endocrine modulating substances that may affect aquatic species, few studies to date have examined this hypothesis. The objective of this pilot study was to investigate potential endocrine modulation caused by lagoon water collected from swine, dairy cattle and beef cattle CAFOs, and aqueous extracts of chicken CAFO waste, using *in vivo* assays in *Xenopus laevis*. We used a tail fin resorption assay in pre-metamorphic tadpoles to evaluate potential thyroid modulation, and evaluated potential sex steroid hormone modulation by determining serum vitellogenin (Vtg), testosterone and 17β-estradiol concentrations in adult male frogs. Depending on the CAFO examined, both delayed and stimulated metamorphosis (time to tail resorption) was observed, suggesting possible CAFO-specific effects on thyroid hormone-mediated development. Significant induction of the yolk protein precursor Vtg in males was observed only in experiments with the chicken CAFO waste. Serum estradiol levels were elevated compared to controls in adult male frogs exposed to discharges collected from dairy and chicken operations. There were no consistent effects of CAFO discharges on serum testosterone levels. The observed variation in amphibian responses may be related to differences among CAFOs in animal gender, pharmaceutical usage, nutrients, bacterial flora, and/or treatment of lagoon wastes.

Changes in Growth, Secondary Sex Characteristics and Reproduction of Fathead Minnows Exposed for a Lifecycle to Bleached Sulphite Mill Effluent. J.L. Parrott[1], C.S. Wood[2], P. Boutot[2] and S. Dunn[3]. [1]Environment Canada, National Water Research Institute, Burlington, ON; [2]NexFor (Noranda Inc.) Technology Centre, Pointe Claire, QC; and [3]Fraser Paper Inc., Edmundston, NB.

During the Cycle 1 Environmental Effects Monitoring (EEM) studies, yellow perch captured downstream of Nexfor Edmundston bleached sulphite mill (BSM) had reduced gonad size and fecundity. Exposures of goldfish to final effluent for 21 days showed decreases in circulating steroid levels. To assess the potential effects of long-term exposure to bleached sulphite mill effluent (BSME), fathead minnow (FHM, *Pimephales promelas*) lifecycle tests were carried out in a flow-through bioassay trailer at the mill secondary treatment lagoons. Exposure concentrations included 0, 1, 3, 10, 30, 50 and 100% final effluent with ethinylestradiol (EE2, 10 ng/L) as a positive control compound. Fertilized FHM eggs were hatched in effluent and monitored through to 30, 60 and 125 days post-hatch.

The effluent caused a significant increase in the growth of fish, and this effect could be seen in as little as 30 days at exposure concentrations of 3% BSME and above. At 60 days post-hatch, one of the earliest endocrine-disruption (ED)-specific endpoints was the premature development of ovipositors in fish exposed to 30% BSME. Changes in external sex characteristics and reproduction were very sensitive endocrine-disruption (ED)-specific endpoints, and required four months of exposure. At maturity, fish had changes in secondary sex characteristics, with significantly increased ovipositor index (a feminization) of male fish in effluent concentrations above 30%. Higher effluent concentrations resulted in a majority of fish that looked externally like females. At low BSME concentrations (3%) we saw some female fish that had male sex characteristics (this was not seen in control fish). This masculinization of female fish was statistically significant at 10% effluent. A very sensitive endpoint and probably the most meaningful change observed was a decrease in reproduction. Fish exposed to 1 to 3% BSME produced a similar number of eggs to control fish; however, exposure to 10% BSME reduced egg production by over 80%. Fish exposed for an entire lifecycle to 30% BSME and above failed to produce any eggs. Concentrations of final effluent in the Saint John River range from less than 1% to 10%, depending on the season and river flow. The research demonstrates the feasibility and potential usefulness of on-site flow-through fish lifecycle exposures for the assessment of pulp mill final effluents.

Regulatory Limitations on the Application of Biocides by Rapid Response Action Plans to Control or Eradicate Recently Introduced Exotic and Invasive Aquatic Species. A.J. Niimi. Department of Fisheries and Oceans, Bayfield Institute, Burlington, Ontario.

A number of North American water bodies have been severely impacted by invasive exotic species. Even native species can become problematic when community-structure relationships are destabilised to a level where an aggressive species can assume a dominant role in an aquatic ecosystem. The rapid increase in exotic species introductions on a global scale over the past few decades has increased the realisation that little can done once a species becomes well established.

Various agencies are currently developing Rapid Response Action plans to deal with recently discovered species.

Control and eradication may be possible where the distribution of the undesirable species is limited to areas where physical and hydrological conditions to not impede treatment efforts. Some biocides have been well tested, and information is available on their potency and environmental fate. Chemicals like rotenone have been approved for use to control fish, but species of concern now includes microbes, invertebrates, vertebrates and plants. A large number of toxicants with difference modes of action could be needed to deal with differences in effective concentrations within and between taxonomic groups. Some biocides are now regulated by agencies where approval permits are based on current use patterns, and intended use for control and eradication purposes could require substantially larger quantities. Seeking permission to conduct a treatment program is a poorly defined variable because of uncertainties about how many regulations would be applicable and jurisdictional issues about the water body to be treated. Time required to obtain approval is critical because there are limits on when treatment should be applied to achieve a high probability of success. Examples will be presented where treatment was applied within one week after a decision was made to eradicate, and others where many months were required where public hearings was part of the decision making process.

Cyanobacteria – an *in vitro* Approach to Studying their Toxic Potential to Fish. K. Schirmer and I. Teneva. Junior Research Group of Molecular Animal Cell Toxicology, UFZ Centre for Environmental Research Leipzig-Halle, Leipzig, Germany.

Toxins produced by cyanobacteria (blue-green algae) have been reported in marine- as well as fresh-water environments throughout the world. Improved detection methods and increased occurrence of cyanobacterial blooms due to eutrophication of many waterbodies are raising awareness of cyanobacterial toxins as a potential risk to environmental health. Most studies focus on the well-known hepatotoxin, microcystin. However, toxic effects elicited by cyanobacterial extracts can often not be explained by microcystin alone, thereby highlighting the importance of biological methods for detecting adverse effects.

We explored the application of the rainbow trout liver cell line, RTL-W1, to investigate the freshwater cyanobacterium *Lyngbya aerugineo-coerulea* for its potential to affect fish health. Despite the absence of microcystins, significant cytotoxic responses were observed upon exposure to the cyanobacterial extracts of the fish cells. Patterns of toxicity varied between intra- and extracellular extracts, indicating that compounds released by cyanobacteria differed from those accumulated internally. Another determinant of toxicity was temperature. Overall, the results suggest that the piscine *in vitro* system is useful for screening cyanobacteria for their potential toxicity to fish. Combined with an effect-directed chemical analysis, the *in vitro* approach can be used in the future to identify the as of yet unknown toxic *Lyngbya* metabolites in particular and to screen cyanobacterial extracts for their toxicity in general.

Ecotoxicological Investigations with the ^{15}N Ecotoxicological Stable Isotope Metabolic Assay in Plant Systems. K. Jung, Ch. Hafner, E. Schmidt and G. Schüürmann. Department of Chemical Ecotoxicology, UFZ Centre for Environmental Research, Leipzig, Germany.

The characterization and assessment of toxicity of complex environmental samples and chemical substances necessitates the usage of biological assay techniques. Apart from tests measuring lethal effects of toxicants, increasing attention is given to the development of techniques for the detection of sublethal toxicity, e.g., depression of metabolic states. In view of the increasing number of new substances and contaminated environmental samples, new test procedures should be rapid, sensitive and reproducible.

The stable isotope ^{15}N test (ESIMA = Ecotoxicological Stable Isotope Metabolic Assay) was developed as a tool to asses biological effects of chemicals and contaminated environmental samples on plant metabolism. The test measures the effect of toxicants on the incorporation of ^{15}N labelled tracer ($^{15}NH_4^+$, $^{15}NO_3^-$) into the total N-fraction (both the non-protein and protein fraction) of plants. As plant materials segments of *Pisum* epicotyls and seedlings from cress *(Lepidium sativum)* are used because their high metabolic activity. The plant material is incubated in a tracer/pollutant medium under standard conditions in comparison to controls for some hours; subsequently, the ^{15}N incorporation (^{15}N abundance; atom-%^{15}N) is analysed emissions spectrometrically. Specificity and sensitivity of effects as indicated by ESIMA are compared to effects as measured by established ecotoxicological bioassays. The results of the study clearly indicate the suitability of ESIMA for assessing toxic impacts on metabolic processes.

Encouraging and Discouraging Plant Uptake of Metals from Contaminated Soils: A Review of Field Trials in Support of Developing Risk Management Options for Remote Lightstations. C.J. LaCoste[1] and N.J. Healey[2]. [1]Jacques Whitford Environment Limited, Vancouver, BC; and [2]Canadian Coast Guard, Victoria, BC.

Pilot scale phytostabilisation and phytoextraction field trials were established at two lightstations on the Pacific coast. The principal contaminant of concern is lead (mean=2,500 mg/kg); however, remediation of Cd, Cu, Hg and Zn is also of interest. Phytostabilisation - Two treatments (bonemeal and limestone) and a control plot were used to determine the efficacy of reducing phytoavailability of metals to garden produce. Six plant species were grown and analyzed. Co-located soil samples were collected to determine uptake factors. A semi-quantitative analysis revealed that bonemeal and limestone treatments decreased Pb uptake by up to a factor of three. Phytoextraction - Three plant species, *Pisum sativum, Brassica juncea,* and *Helianthus annus,* and two soil amendments, Ethelynediaminetetraacetate (EDTA) and citric acid, were used to determine potential for phytoextraction of metals from contaminated soils. Six plots were constructed and lined with polypropylene and geotextile. EDTA (10 mmol/kg) and citric acid (2 mmol/kg) were applied approximately 7 days prior to harvest of above ground biomass. Plot leachate was collected for analysis prior to addition of chelators and at the time of harvest. The efficacy of each of the treatments will be evaluated to determine if phytoremediation is a feasible remediation or risk management option for these sites.

Photo-Transformation and Photo-Activation of Select Ordnance Compounds. R.S. Carr[1], M. Nipper[2], J.M. Biedenbach[1], R.L. Hooten[1] and K. Miller[3]. [1]U.S. Geological Survey, Corpus Christi, TX; [2]Texas A&M University-Corpus Christi, Corpus Christi, TX; and [3]Navy Facilities Engineering Service Center, Port Hueneme, CA.

Previous studies indicated that several ordnance compounds (nitroaromatic and nitroamines) in sea water were acutely and chronically toxic to marine organisms (Carr et al., SETAC-Europe, 1999), but very little information is available on the influence of ultraviolet (UV) radiation on the toxicity of these compounds. Based on past studies, picric acid (trinitrophenol) and 2,6-dinitrotoluene (2,6-DNT) were selected for photo-transformation and photo-activation studies in marine waters. HPLC measurements of seawater solutions exposed to simulated solar radiation (SSR) over a 6 week period indicated that picric acid was not photo-transformed whereas 2,6-DNT started to photo-transform immediately and none of the original compound was left after 72 hours under SSR. The identity of the primary photo-transformation product of 2,6-DNT is being determined by GC-MS analysis and its toxicity is being assessed. Similar to the effect of SSR on the photo-transformation, no photo-induced toxicity (photo-activation) was observed for picric acid in the sea urchin fertilization test, whereas the toxicity of 2,6-DNT was strongly enhanced. Further assessments of the photo-activation of 2,6-DNT after more prolonged exposures to the parent compounds prior to exposure to SSR are under way. Results of tests performed with benthic copepods and meiofaunal polychaetes will also be presented.

Bioaccumulation and Chronic Toxicity of Uranium at Different Life Stages of the Aquatic Invertebrate *Chironomus tentans*. J. Muscatello and K. Liber. Toxicology Centre, University of Saskatchewan, Saskatoon, SK.

Northern Saskatchewan is home to some of the top-producing uranium (U) mines in the world. Consequently, high U concentrations can be found in water and sediment in areas downstream at these mines, suggesting that these are important areas of potential impact of U releases to the environment. Benthic invertebrates are likely among the most highly exposed organisms due to the capacity of sediments to accumulate U. Tolerance of invertebrates to elevated concentrations of metals can involve the exclusion, active excretion, or intracellular storage of those metals. Therefore, to predict the effects of U on aquatic invertebrates, it is important to determine the relationships between U exposure concentrations and resultant concentrations in biological tissues. The objectives of the present study were: [1] to determine what concentrations of U in water can affect survival, growth and adult emergence of *C. tentans*, [2] to establish whether tissues involved in storage, excretion, ingestion and ion regulation in this aquatic invertebrate were sites of U accumulation, and [3] to determine whether metamorphosis can affect U mobility or excretion in this organism. Results to date indicate that chronic exposure to sublethal U concentrations produces larval growth retardation and adult emergence reductions in *C. tentans*. In addition, U that accumulated during *C. tentans* larval stages was partially excreted during metamorphosis to the adult stage. The distribution of U within *C. tentans* larvae, pupae and adults is presently being mapped using x-ray microprobe analysis.

Assessing the Toxicity of Surfactants to Protozoa, Fish and Mammalian Cells. V.R. Dayeh[1], S. Chow[1], K. Schirmer[2], D.H. Lynn[3] and N.C. Bols[1]. [1]Department of Biology, University of Waterloo, Waterloo, ON; [2]UFZ Centre for Environmental Research Leipzig-Halle, Germany; and [3]Department of Zoology, University of Guelph, Guelph, ON.

Synthetic surfactants, such as Triton X-100, are found throughout the environment. Human exposure occurs through their presence in cosmetical, pharmaceutical and dermatological formulations; environmental exposure results from their release through the activities of several industries, including the pulp and paper and textile industries. In this work, the toxicity of Triton X-100 was explored at the cellular level with cell lines from humans, such as HepG2 and Caco-2, from rat, H4IIE, and from rainbow trout, such as RTL-W1 and RTgill-W1, and with the free living protozoan, *Tetrahymena thermophila*. Several cellular parameters were investigated as possible endpoints of cytotoxicity and as indicators of potential cytotoxic mechanisms, which were assayed with fluorescent indicator dyes on cells in microwell cultures. Alamar Blue™ was used for metabolic activity, propidium iodide and CFDA-AM for plasma membrane integrity, and neutral red for lysosomal function. Alamar Blue™ was the indicator dye that could be applied most conveniently to all cells and provided the most consistent results. Fish cells were the most sensitive to surfactant exposure. For cell bioassays of surfactants in environmental samples, Tetrahymena appear to be the most promising cell.

Assessment of Sensitivities of Two Populations of the Amphipod, *Eohaustorius estuarius.*

D.L. Lee[1], K.G. Doe[2] and G.C. van Aggelen[3]. [1]Environment Canada, Ocean Disposal Control Program, North Vancouver, BC; [2]Environment Canada, Environmental Science Centre, Moncton, NB; and [3]Environment Canada, Pacific Environmental Science Centre, North Vancouver, BC.

Eohaustorius estuarius is an amphipod species approved and used by Environment Canada to evaluate sediment quality. Organisms are normally obtained from Oregon, USA, however,populations of this amphipod species has been identified on the west coast of Vancouver Island and sediment testing using the Canadian population was recently undertaken by the Disposal at Sea program. While comparative sensitivities and tolerance ranges between approved test species has been investigated by Environment Canada researchers in the past, the range of sensitivities between populations of the same species has not been previously investigated. Given that this species and the Environment Canada test method is used in the Disposal at Sea regulatory framework under the authority of Part 7, Division 3 of the *Canadian Environmental Protection Act* (CEPA), studies were initiated by Environment Canada: [1] to determine whether populations of *E. estuarius* identified from the west coast of Vancouver Island are viable for testing purposes, [2] determine whether the British Columbian population of *E. estuarius* showed the same degree of sensitivities and/or responses as the Oregon state *E. estuarius* population, and [3] determine if the same regulatory conclusions could be drawn from different populations of the same test species. The preliminary results of these studies are presented.

BEST STUDENT PAPER AWARDS / PRIX POUR LES MEILLEURS EXPOSÉS PAR DES ÉTUDIANTS

Best Platform Paper

Christy A. Morrissey. Department of Biological Sciences, Simon Fraser University, Burnaby, BC. Migration within a Watershed Influences the Contaminant Profiles of American Dippers.

Other Notable Platform Papers

Joline R. King. Department of Biological Sciences, Simon Fraser University, Burnaby, BC. The Role of Bacterial versus Sediment Sources in Mediating Metal Bioavailability to the Bivalve *Mytilus trossulus*. CAEAL Award.

Andrea Lister. Department of Zoology, University of Guelph, Guelph, ON. Waste Products of Oilsands Mine Inhibit Sex Steriods in Exposed Fish.

Jennie R. Christensen. Forest Sciences Centre, University of British Columbia, Vancouver, BC. The Amphibian Sperm Inhibition Toxicological Test Method.

Best Poster Paper

Deborah E. Ratzlaff. Department of Biological Sciences, Simon Fraser University, Burnaby, BC. The Role of Aqueous Bioavailability in the Bioconcentration of Phthalate Esters.

Other Notable Poster Papers

Michelle E. Bowerman. Queen's University, Kingston, ON. Reduced Survival of Early Life Stage Fish Exposed to PAH-Contaminated Sediment. CAEAL Award

Jorgelina Muscatello. Toxicology Centre, University of Saskatchewan, Saskatoon, SK. Bioaccumulation and Chronic Toxicity of Uranium at Different Life Stages of the Aquatic Invertebrate *Chironomus tentans*.

Glenys M. Webster. School of Resource and Environmental Management, Simon Fraser University, Burnaby, BC. Dietary Uptake, Internal Distribution and Metabolism of Phthalate Esters in Staghorn Sculpin (*Leptocottus armatus*).

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WORKSHOP PROCEEDINGS / COMPTE RENDUS D'ATELIER

The Proceedings of each Annual Aquatic Toxicity Workshop have been published in a series of Technical Reports listed below. These Proceedings are generally provided to each Workshop participant, and are also sent to selected libraries, government departments and other agencies. Copies of 4th and subsequent Proceedings may be available for a charge, as photocopies or fiche, from Micromedia Limited, 240 Catherine Street, Suite 305, Ottawa, ON, K2P 2G8 (613-237-4250).

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